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Section 3001 and 250.14-Infectious Waste
Background Document

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This document provides background information and support for regulations which have been designed to identify and list hazardous waste pursuant to Section 3001 of the Resource Conservation and Recovery Act of 1976. This document presents the Agency's rationale in determining the definition of infectious hazardous waste.

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BACKGROUND DOCUMENT

RESOURCE CONSERVATION AND RECOVERY ACT
SUBTITLE C - HAZARDOUS WASTE MANAGEMENT

SECTION 3001 - IDENTIFICATION AND LISTING OF HAZARDOUS WASTE

SECTION 250.14 - HAZARDOUS WASTE LISTS

INFECTIOUS WASTE

DECEMBER 15, 1978

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF SOLID WASTE

This document provides background information and support for regulations which have been designed to identify and list hazardous waste pursuant to Section 3001 of the Resource Conservation and Recovery Act of 1976. It is being made available as a draft to support the proposed regulations. As new information is obtained, changes may be made in the background information and used as support for the regulations when promulgated.

This document was first drafted many months ago and has been revised to reflect information received and Agency decisions made since then. EPA made some changes in the proposed regulations shortly before their publication in the Federal Register. We have tried to ensure that all of those decisions are reflected in this document. If there are any inconsistencies between the proposal (the preamble and the regulation) and this background document, however, the proposal is controlling.

Comments in writing may be made to:

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Draft Background Document

Hazardous Waste Identification and Listing

Infectious Waste

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Appendix

Draft Background Document
Hazardous Waste Identification and Listing
Infectious Waste

3.1 Introduction

The purpose of this chapter of the background document is to present the Agency's rationale in determining the definition of infectious hazardous waste.

To date it has been the policy of the Agency under Section 3001 of the Act, to define chemical and physical hazardous waste characteristics such as toxicity, flammability, and corrosivity, in quantitative terms; i.e. criteria have been chosen that best quantify each hazardous characteristic, with certain hazard levels specified for each tested parameter (e.g., flashpoint for flammability, pH for corrosivity). For enforcement purposes, this method of quantitatively defining a hazardous waste is most desirable. It would follow then, that a similar type of definition for "infectious characteristics" would be the most useful one from a regulatory point of view.

Unfortunately, such quantification of infectious characteristics is not possible, as will be discussed in this document. Instead of specifying a certain number of infectious agents allowed to be present in a waste, the Agency has chosen to define infectious waste by specifying the sources where disease microorganisms may occur. After consultation with experts in the public health field and consideration of current State regulatory programs, the Agency has reached the conclusion that such source identification of infectious waste is the most inclusive and enforceable method of regulation.

3.2 Solid Waste/Disease Relationships

Basic principles of epidemiology include a chain of events necessary for the transmission of disease microorganisms. In the case of solid waste, the chain involves the production of solid waste contaminated with disease agents, the transfer of the disease agents from a waste to a host, and the manifestation of the disease in a host. The completion of this chain, or transmission of disease, is dependent upon the optimization of many variables. For example, some variables include the kinds and numbers of disease agents found in the solid waste, the environmental conditions of the solid waste substrate, and the capability of the disease agent to survive. Some variables that affect the host's susceptibility to disease are the manner of contact with the waste, the general health and age of the host, his or her previous contact with the disease agent, and his or her response (clinical versus subclinical) to the disease agent.

To specify a "safe" number of disease organisms allowed in a waste would be to ignore the large number of variables involved in the transmission of disease. Additionally, for certain viral and parasitic diseases, it is known that only one organism, if successfully transmitted, can cause a clinical response in a host; yet for other disease agents it is known that hundreds or even thousands of organisms are necessary. Therefore setting a safe number of organisms for solid waste would involve specifying a safe level for each disease agent and providing a means to analyze for each one. Unfortunately, dose levels for all disease agents are not known at present and methods of environmental sampling and analysis for many disease agents have not been developed.

3.3 Indicator Organisms

Several EPA contacts have suggested the use of indicator organisms such as Salmonella spp., fecal coliforms, or S. aureus as an index of overall (i.e. viral, bacterial, fungal, parasitic) biological hazard of a waste. The problems associated with the use of indicator organisms have been recognized by EPA. For water standards, the Office of Water Program Operations originally suggested the use of fecal coliform as an indicator organism to determine the effectiveness of the chlorination process (40 CFR 133). This standard was later deleted (FR July 26, 1976) (1), with EPA recognizing that fecal coliform is "not an ideal indicator of pathogenic (sic) contamination" but is "a practical indicator of relative disease causing potential."

While microbial concentration standards may be applicable in the evaluation of the efficacy of wastewater treatment systems, their applicability as absolute quality standards remains to be demonstrated. A problem is that in some situations, the die-off or regrowth of indicator organisms does not always parallel that of the disease organisms, the presence of which they are supposed to indicate. For example, it has been found that certain pathogenic viruses are more resistant to conventional wastewater treatment than are the coliforms (Cooper and Golueke, 1977). (2) As such, it has been decided that indicator organisms will not be used for purposes of defining infectious characteristics in this regulation.

3.4 The Source Approach

Ruling out the specification of "safe" microbial contamination levels and the use of indicator organisms, EPA has chosen to specify the solid waste sources where disease-causing organisms are known to occur, and to define waste from these sources as infectious waste.

The disease-causing organisms are, for purposes of this regulation, to be defined by CDC's "Classification of Etiologic Agents on the Basis of Hazard." (3) Sources of waste where Class 1 agents are known to occur are excluded from the definition of infectious waste, since Class 1 agents are of no or minimal hazard under ordinary conditions. Sources where Class 2 (agents of ordinary potential hazard) and up are known to occur are included, since Class 2 agents are disease causing. Descriptions of the CDC Classes used to identify the infectious waste sources are given below.

Class 2

Agents of ordinary potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

Class 3

Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

Class 4

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Class 5

Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

NOTE: It has been pointed out that the current CDC list does not include some agents of significance (e.g. Giardia, Ascaris, Legionnaires bacterium) as well as it does include one non-pathogen (Naegleria gruberi). The reader should keep in mind that the list is periodically revised. The most recently published list would be applicable.

The relationship between the agents in these classes and the waste sources where these agents are found was developed by using information found in the literature and consultation with public health experts. This approach is in agreement with the Center for Disease Control, USPHS, for sources other than health-care facilities waste; in agreement with the Joint Commission on Accreditation of Hospitals for sources of hospital waste; in agreement with NIH for sources of laboratory waste; and with the various State regulatory programs for other sources of infectious waste.

3.5 The Current State Approach

Nine states have defined the infectious characteristics of hazardous waste either wholly or in part. Terms such as "biohazardous," "health-services hazardous," "pathological," "biological," and "hazardous-infectious" are used to describe infectious characteristics of the waste of concern. These examples of State definitions are shown in Table 1.

The definitions are derived from one, or a combination, of four methods: a list of infectious (etiologic) agents; a list of infectious items that have a high probability of being contaminated; a list of sources of infectious waste; or a prose definition. The one list of infectious agents referenced is HEW's list of etiologic agents. Table 2 shows a composite matrix of infectious items and sources of infectious wastes, identifying the States that consider each one.

It is interesting to note that not one of these definitions attempts to quantify numbers of disease organisms that would render a waste infectious and that it is these same States that have promulgated criteria for physical/chemical characteristics of hazardous waste on a quantitative basis similar to the ones EPA is considering. The approach that the Agency is taking to define infectious characteristics of waste, then, and the deviance of this approach from that of defining other characteristics of hazardous waste, is in line with the thinking proposed by the most progressive State hazardous waste management programs.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
California Department of Health		Proposed Revisions to the Code, Title 22	Biohazardous waste (infectious waste) shall be defined as, but is not limited to; (1) Significant laboratory or pathology waste of an infectious or experimental nature which has not been autoclaved including pathologic specimens (which shall include all human parts removed surgically or at autopsy, specimens or blood elements, excreta and secretions obtained from patients) and disposable fomites such as bandages, dressings, casts, catheters, and tubing which has been in contact with wounds, burns or surgical incisions and which are suspect or have been medically identified as biohazardous. (2) Surgical specimens and attendant disposable fomites. (3) Similar disposable material from outpatient areas and emergency rooms. (4) Equipment, instruments, utensils and fomites of a disposable from the rooms of patients with suspected or diagnosed communicable disease requiring isolation. (5) Sharps which include but are not limited to needles, syringes and blades. (6) Dangerous drugs as defined in Sectio 4211 of the Business and Professions Co

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
California Department of Health		Assembly Bill No. 1593: An Act to Amend Section 25116. Ch. 6.5. Division 20, of the Health and Safety Code	"Infectious" means containing pathogenic organisms, or having been exposed, or reasonably being expected to have been exposed, to contagious or infectious disease. Articles which are "infectious" include, but are not limited to, the following: (1) Wastes that contain pathologic specimens, tissues, specimens or blood elements, excreta or secretions from humans or animals at a hospital, medical clinic, research center, veterinary institution, or pathology laboratory. (2) Surgical operating room pathologic specimens and articles attendant thereto which may harbor or transmit pathogenic organisms. (3) Pathologic specimens and articles attendant thereto from outpatient areas and emergency rooms. (4) Discarded equipment, instruments utensils and other articles which may harbor or tranmit pathogenic organisms from the rooms of patients with suspected or diagnosed communicable disease.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Illinois EPA	Environmental Protection Act	Special Waste Land Disposal Disposal Criteria	Industrial Process Effluent - Any liquid, solid, semi-solid or gaseous refuse generated as a direct or indirect result of the creation of a project or the performance of a service, including but not limited to... hospital pathological waste. Hazardous Waste - Any refuse that...is harmful or potentially harmful to human health or the environment...due to its.. pathological...nature.
Maryland Department of Natural Resources	Safe Disposal of Hazardous Substances Act of July 1976	COMAR 08.05.05 Control of the Disposal of Designated Hazardous Substances Regulations .01-.18 .18, Designated Hazardous Substances, Class III, B (4)	A "Designated Hazardous Substance" includes pathological and medical wastes from hospitals, laboratories, and similar operations.

TABLE -

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
State of Maryland Department of Health and Mental Hygiene		Proposed Regulations for Medical Waste Disposal; "Subcommittee Report to the Task Force on Medical Waste Disposal - December 6, 1976"	The term medical wastes, encompassing materials hitherto called "infectious" "pathological", "contaminated", "special", and "hazardous" shall be replaced with the following new terms: (1) Hospital Medical Wastes - shall mean all solid waste generated within a hospital. Blood and blood products shall be included in this solid waste category. (2) Nursing Home Medical Wastes - shall be defined in two categories, as follows: (a) All disposable fomites from isolation areas, all dressings, pledgets, swabs, tongue depressors, plaster casts, body tissues, laboratory wastes, needles, syringes, I.V. apparatus, and medications (as permitted under Federal, State and local regulations). (b) Additional items which may be included in the above category include diapers and perineal pads.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Pollution Control Agency Division of Solid Waste	Minnesota Statutes 1971: Chapters 115, 116,400,473D	Solid Waste Disposal Regulations, Section SW-1	Hazardous Infectious Waste - Waste originating from the diagnois, care or treatment of a person or animal that has been or may have been exposed to a contagious or infectious disease. Hazardous infectious waste includes, but is not limited to, (1) All wastes originating from persons placed in isolation for control and treatment of an infectious disease. (2) Bandages, dressings, cases, catheters, tubing, and the like, which have been in contact with wounds, burns, or surgical incisions and which are suspect or have been medically identified as hazardous. (3) All anatomical waste, including human and animal parts of tissues removed surgically or at autopsy. (4) Laboratory and pathology waste of an infectious nature which has not been autoclaved. (5) Any other waste, as defined by the State Board of Health, which, because of its hazardous nature, requires handling and disposal in a manner prescribed for (1) through (5).

TABLE -

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Department of Health, Health Facilities Division		Interpretive Policies for the Physical Plant: Handling and Disposal of Infectious Waste (Current DOH Guidelines)	Infectious Waste: (1) Hazardous Infectious Waste (same as above). (2) General Infectious Waste (contaminated): (a) Bandages, dressing, casts, catheters tubing, and the like, which have in contact with wounds, burns, or surgical incisions, but are not suspected or have been not medically identified as being of a hazardous infectious nature. (b) Discarded hypodermic needles and syringes, scalpel blades, and similar materials, when suspected or identified to be of a hazardous infectious nature. (c) Incinerator ashes from infectious waste.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Pollution Control Agency		Proposed, but to no longer be part of the hazardous waste regulations, HW-1	Health services hazardous wastes: wastes that originate from the diagnois, care, or treatment of a human or an animal, and wastes of similar composition, excluding animal or human corpses but including: (1) Laboratory wastes, including: (a) Pathological specimens: tissues and specimens of blood elements, excreta, and secretions obtained from patients. (b) Infectious cultures: cultures that have been used in the detection, maintenance, or isolation of infectious organisms or suspected infectious organisms including, but not limited to microorganisms and helminths capable of producing infection or infectious disease. (c). Disposal fomites: any waste that may harbor or transmit infectious organisms.

TABLE -

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Pollution Control Agency, (CONT.)		Proposed, but to no longer be part of the hazardous waste regulations, HW-1	(2) Surgical and obstetrical wastes, pathological specimens, and disposal fomites from surgical operating rooms, outpatient areas, emergency rooms and similar areas where such wastes are generated. (3) Equipment, instruments, utensils, and fomites of a disposable nature from the rooms of patients with suspected or diagnosed communicable disease, or from the rooms of patients who by nature or their disease are required to be isolated by the State Board of Health. (4) Hypodermic needles and syringes, scalpel blades, suture needles and similar materials. (5) Mixtures of any of the wastes in (1) through (5) and other wastes that have been collected within the same container.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
NSWMA		Gaynor Ward Dawson Draft of Model Criteria for Hazardous Waste	Materials or wastes which are capable of transmitting infectious diseases at a probability level above that from daily life should be defined as hazardous wastes.
New York Department of Environmental Conservation	6 NYCRR Part 360, Solid Waste Management Facilities		Criteria for identifying Hazardous Substances: Infectious: Materials containing infectious agents which are capable of causing death or severe illness or which are highly contagious.
Oregon Department of Environmental Quality	Oregon Laws 1971 (HB 1051), Chapter 648	DEQ 41, Chapter 340	"Hazardous Solid Waste" includes "infectious", but infectious not defined.

TABLE

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Pennsylvania Department of Environmental Resources	Pennsylvania Solid Management Act (35 (35 PS6-001), PL 241	Hazardous Waste Management Profile	General Classification of Hazardous Wastes (1) Pathogenic Materials (a) biological solids (b) laboratory wastes (c) infectious wastes (2) Other Hazardous Solid Waste (a) diseased animals
Texas Department of Health Resources		Comments to ANPR	Hazardous biological waste should include all pathological waste from chemical biological and contagious wards as well as animals dead of unknown disease and unstabilized domestic sewage.
State of Washington Department of Ecology		Washington Administrative Code (WAC) Hazardous Waste Regulation, Chapter 173-302 WAC	Waste containing etiologic agents are toxic dangerous wastes. Etiologic agent means a viable microorganism or its toxin, which causes or may cause human disease, and is limited to those agents listed in 42 CFR 72.25(c) of the regulations of the Department of HEW.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Ontario, Department of the Environment, Waste Management Branch			Pathological waste - Waste resulting from the discard of tissue or of material or equipment subject to contamination with infectious organisms.
Ontario, Department of the Environment, Air Management Branch			Pathological Waste - Carcasses, human and animal, solid organic wastes from hospitals, laboratories, abattoirs, and animal compounds, disposable operating theatre garments and swabs, maternity and incontient pads, disposable diapers, and other similar items which might contain pathogenic bacteria.

Table 2A.

Areas/Sources Identified as Sources of Infectious Wastes, By State

	California	Illinois	Maryland	Minnesota	New York	Oregon	Pennsylvania	Texas	Washington	Ontario
Abattoir										X
Animal Compounds										X
Veterinary Hospitals	X									
Health Services				X						
Hospital, "pathological waste"	X	X	X							X
Emergency Rooms	X			X						
Isolation Rooms	X		X	X				X		
Laboratory	X		X	X			X			X
Outpatient Areas	X									
Pathology Laboratory	X									
Surgical Operating Room	X			X						
Medical Clinics	X									
Nursing Homes			X							
Research Center	X									
Sewage Sludge								X		

Table 2B.

Items Identified, By State

	California	Illinois	Maryland	Minnesota	New York	Oregon	Pennsylvania	Texas	Washington	Ontario
Autopsy Specimens	X			X						
Blood Specimens	X		X	X						
Excreta	X			X						
Human Carcasses										X
Obstetrical Waste				X						X
Pathologic Specimens	X			X						
Secreta	X			X						
Surgical Specimens	X			X						
Tissues	X		X	X						
Etiologic (infectious) Agent-containing items					X				X	X
Attendant Disposable Fomites	X		X	X						X
Disposable Diapers			X							X
Instruments (disposable)	X			X						
I.V. Apparatus			X							
Perineal Pads			X							
Sharps	X		X	X						
Utensils	X			X						
Dangerous Drugs	X		X							

Table 2 B (Cont.)

Items Identified, By State

	California	Illinois	Maryland	Minnesota	New York	Oregon	Pennsylvania	Texas	Washington	Ontario
Biological Solids						X				
Incinerator Ash From Infectious Waste			X							
Diseased Animals			X			X	X		X	

3.6 Related Federal Regulations and Guidelines

No current federal regulations specifically address the problem of infection as related to solid waste. The Department of Transportation has published Interim Hazardous Materials Regulations (49 CFR Parts 171-177)(4) in which "etiologic agent" is defined (173.386) for purposes of commodity transport. The definition reads as follows:

> § 173.386 Etiologic agents; definition and scope.
>
> (a) *Definition*. For the purpose of Parts 170-189 of this subchapter:
>
> (1) An "etiologic agent" means a viable microorganism, or its toxin, which causes or may cause human disease, and is limited to those agents listed in 42 CFR 72.25(c) of the regulations of the Department of Health, Education, and Welfare.

HEW's list (5) consists of the following etiologic agents.

BACTERIAL AGENTS

Actinobacillus--all species.
Arizona hinshawii--all serotypes.
Bacillus anthracis.
Bartonella--all species.
Bordetalla--all species.
Borrelia recurrentis, *B. vincenti*
Brucella--all species.
Clostridium botulinum, *Cl. chauvoei*, *Cl. haemolyticum*, *Cl. histolyticum*, *Cl. novyi*, *Cl. septicum*, *Cl. tetani*.
Corynebacterium diphtheriae, *C. equi*, *C. haemolyticum*, *C. pseudotuberculosis*, *C. pyogenes*, *C. renale*.
Diplococcus (Streptococcus) pneumoniae.
Erysipelothrix insidiosa.
Escherichia coli, all enteropathogenic serotypes.
Francisella (Pasteurella) tulcrensis.

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Haemophilus ducreyi, H. influenzae.
Herellea vaginicola.
Klebsiella--all species and all serotypes.
Leptospira interrogans--all serotypes.
Listeria--all species.
Mima polymorpha.
Moraxella--all species.
Mycobacterium--all species.
Mycoplasma--all species.
Neisseria gonorrhoeae, N. meningitidis.
Pasteurella--all species
Pseudomonas pseudomallei.
Salmonella--all species and all serotypes.
Shigella--all species and all serotypes.
Sphacrophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema careteum, T. pallidum, and T. pertenue.
Vibrio fetus, V. comma, including biotype El Tor, and V. parahemolyticus.
Yerscnia (Pasteurella) pestis.

FUNGAL AGENTS

Actinomycetes (including Nocardia species, Actinomyces species and Arachnia propionica).
Blastomyces dermatitidis.
Coccidioides immitis.
Cryptococcus neoformans.
Histoplasma capsulatum.
Paracoccidioides brasiliensis.

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

Adenoviruses--human--all types.
Arboviruses.
Coxiella burnetii.
Coxsackie A and B viruses--all types.
Cytomegaloviruses.
Dengue virus.
Echoviruses--all types.
Encephalomyocarditis virus.
Hemorrhagic fever agents, including Crimean hemmorrhagic fever (Congo), Junin, and Machupo viruses, and others as yet undefined.

Hepatitis-associated antigen.
Herpesvirus--all members
Infectious bronchitis-like virus.
Influenza viruses--all types.
Lassa virus.
Lymphocytic choriomeningitis virus.
Marburg virus.
Measles virus.
Mumps virus.
Parainfluenza viruses--all types.
Polioviruses--all types.
Poxviruses--all members,
Psittacosis-Ornithosis-Trachoma-Lymphogranuloma group of agents.
Rabies virus--all strains.
Reoviruses--all types.
Respiratory syncytial virus.
Rhinoviruses--all types.
Rickettsia--all species.
Rubella viruses--all types.
Simian virus.
Tick-borne encephalitis virus complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses.
Vaccinia virus.
Varicella virus.
Variola major and Variola minor viruses.
Vesicular stomatis virus.
Yellow fever virus.

Comments addressing this Interim Regulation are filed in DOT's Docket HM-142. Many responses suggest that the definition of etiologic agent be expanded to include agents harmful to plants and animals. DOT has not yet published a response to comments.

In considering the possibility of adopting this regulation for defining infectious waste, EPA was concerned with the enforceability of such a list because wastes cannot be adequately tested. EPA would prefer to rely on such a list as a way to identify sources that may contain these etiologic agents. The CDC "Classification of Etiologic Agents on the Basis of Hazard," a more complete list which includes animal etiologic agents, will be used for source-identification purposes. (See Appendix VI of the regulation.)

EPA has previously defined infectious waste in "Guidelines for Thermal Processing and Land Disposal of Solid Waste," FR, August 14, 1974.(6) The definition, which is reprinted below, is felt to be unenforceable, as are most State definitions of infectious waste. Items specified in this definition would be included in the "sources," under the proposed approach. Also, this definition ignores the sewage sludge problem.

> "Infectious waste" means: (1) Equipment, instruments, utensils, and fomites of a disposable nature from the rooms of patients who are suspected to have or have been diagnosed as having a communicable disease and must, therefore, be isolated as required by public health agencies; (2) laboratory wastes such as pathological specimens (e.g., all tissues, specimens of blood elements, excreta, and secretions obtained from patients or laboratory animals) and disposable fomites (any substance that may harbor or transmit pathogenic organisms) attendant thereto; (3) surgical operating room pathologic specimens and disposable fomites attendant thereto and similar disposable materials from outpatient areas and emergency rooms.

3.7 Epidemiological Evidence

In 1967, for the first time solid waste was thoroughly investigated as a reservoir for infectious microorganisms. Thrift G. Hanks, M.D., completed an exhaustive study entitled Solid Waste/Disease Relationships: A Literature Survey. (7) Routes of transmission of human disease from solid waste were described as "pathways," (see diagram below), and all evidence from the literature on solid waste/disease correlation was brought together. Hanks summarizes his findings with the following statement:

> The literature fails to supply data which would permit a quantitative estimate of any solid waste/disease relationship. The circumstantial and epidemiologic information does support a conclusion that, to some disease, solid wastes bear definite, if not well defined, etiologic relationship. The diseases so implicated are infectious in nature; no relationship can be substantiated for noncommunicable disease agents associated with solid wastes, not because of negating data, but because of lack of data.

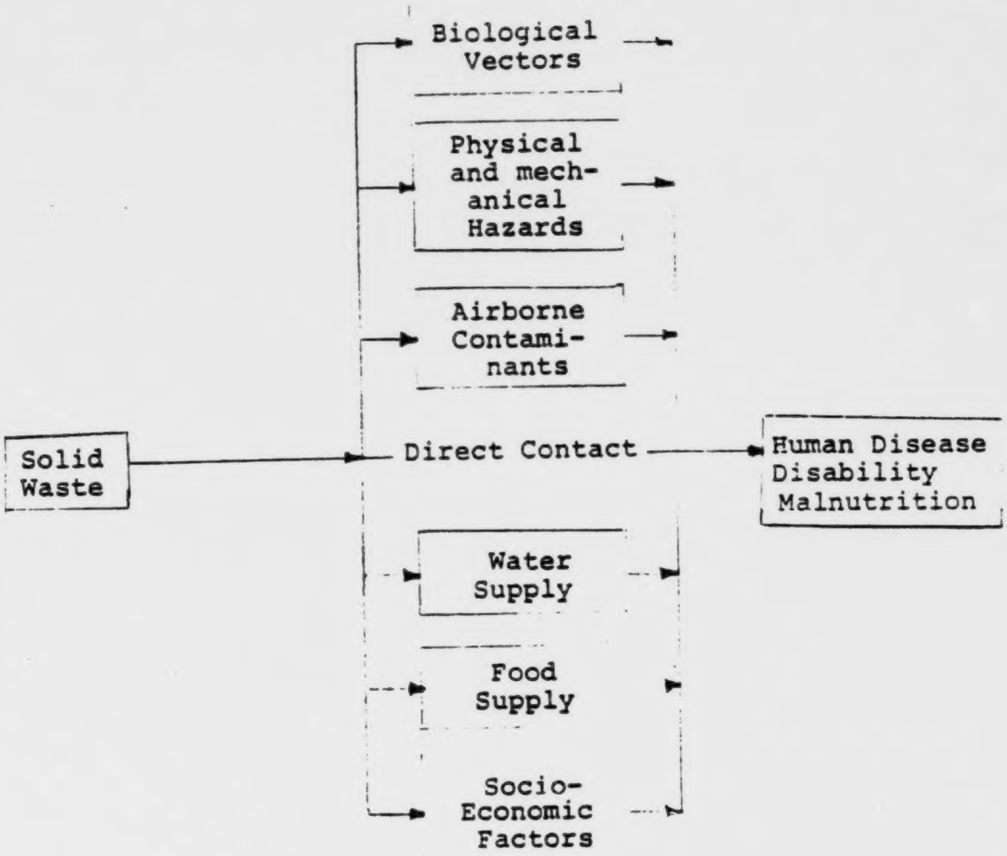
Biological Vectors
Physical and mechanical Hazards
Airborne Contaminants
Solid Waste
Direct Contact
Human Disease Disability Malnutrition
Water Supply
Food Supply
Socio-Economic Factors

Figure 1. T.G. Hanks' Postulated Solid Waste/Human Disease Pathways

There appears to be a paucity of epidemiological data on the subject mainly because funds have never been appropriated for gathering such data. It has not been until recently that EPA has undertaken any epidemiologic studies related to solid waste, which will be completed in several years. Until then, regulation must be based on the microbiological data from studies of the various sources of waste, and on the principles of epidemiology and solid waste-disease relationships.

3.8 Sources Identified

For purposes of defining infectious waste, the sources of these wastes have been identified in the regulation by SIC number with the corresponding industry. These sources are regrouped here for discussion purposes in this document under the following headings:

3.10 Rationale for Regulation of Health Care Facilities Waste
  - Hospitals
  - Veterinary Hospitals

3.11 Rationale for Regulation of Laboratory Waste

3.12 Rationale for Regulation of Unstabilized Sewage Treatment Plant Sludge

Certain departments

3.9 Definitions (8, 9, 10)

For clarification the later discussions, the following definitions are provided:

ANIMAL WASTE - Waste generated from animal care or use; including bedding, egestion, excretions, secretions, tissue, remains, and any inedible by-products of animal processing for food and fiber-production.

AUTOCLAVE - An apparatus for effecting sterilization by steam under pressure. It is fitted with a gauge and a mechanical system which automatically regulates the pressure and the temperature to which the contents are subjected.

BACTERIA - Any of numerous unicellular microorganisms of the class Schizomycetes, occuring in a wide variety of forms, existing either as free-living organisms or as parasites, and having a wide range of biochemical, sometimes pathogenic, properties.

ENTERIC - of or within the intestine.

ETIOLOGIC AGENT - A viable microorganism or its toxin which causes, or may cause human disease. In the case of DOT Regulations, etiologic agents are (or are suspected to be) in relatively small concentrated samples which are shipped to special laboratories for identification

FOMITE - An inanimate object such as an article of clothing, a dish, a toy, or a book, that is not itself corrupted but is able to harbor pathogenic organisms which may by that means be transmitted to others.

FUNGUS - Any of numerous plants of the division or subkingdom Tallophyta, lacking chlorophyll, ranging in form from a single cell to a body mass of branched filamentous hyphae that often produce specialized fruiting bodies, and including the yeasts, molds, smuts, and mushrooms.

INFECTION - The entry and development or multiplication of an infectious agent in the body of man or animal. Infection is not synonymous with infectious disease; the result may be inapparent or manifest. The presence of living infectious agents on exterior surfaces of the body or upon articles of apparel or soiled articles is not infection, but rather is contamination of such surfaces and articles.

INFECTIOUS AGENT - An organism, mainly microorganisms (bacterium, protozoan, spirochete, fungus, virus, rickettsia, or other) but including helminths, capable of producing infectious disease.

INFECTIOUS DISEASE - A disease of man or animal resulting from an infection.

PATHOGEN - An organism capable of producing disease.

PATHOLOGICAL WASTE - Tissues, parts, and organs of humans and animals.

PROTOZOAN - Any of the single-celled, usually microscopic organisms of the phylum or subkingdom Protozoa, which includes the most primitive forms of animal life.

RICKETTSIA - Any of various microorganisms of the genus *Rickettsia*, carried as parasites by many ticks, fleas, and lice. Transmitted to man, they cause diseases such as typhus, scrub typhus, and Rocky Mountain spotted fever.

SOLID WASTE - Any garbage, refuse, sludge from a waste treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, including solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining, and agricultural operations, and from community activities, not including solid or dissolved material in domestic sewage, or solid or dissolved material in domestic sewage, or solid or dissolved materials in irrigation return flows or industrial discharges which are point sources subject to permits under section 402 of the Federal Water Pollution Control Act, as amended (86 Stat. 880), or source, special nuclear, or byproduct material as defined by the Atomic Energy Act of 1954, as amended (68 Stat. 923).

SEWAGE Sludge - The residue resulting from wastewater treatment.

SPIROCHETE - Any of various slender, nonflagellated, twisted microorganisms of the order spirochaetales, many of which are pathogenic, causing syphilis, relapsing fever, yaws, and other diseases.

SURGICAL AND AUTOPSY WASTE - Waste that includes tissue, limbs, organs, placentas, and similar types of materials; synonomous with pathogenic waste.

VIRUS - Any of the various submicroscopic pathogens consisting essentially of a core of a single nucleic acid surrounded by a protein coat, having the ability to replicate only inside a living cell.

ZOONOSIS - An infection or infectious disease transmittable under natural conditions from vertebrate animals to man.

3.10 Rationale for Regulation of Health Care Facilities Waste

The nature of waste generated by health care facilities is of concern to EPA due to a certain amount of potentially disease-contaminated materials found in the waste that are not normally found in other institutional solid wastes. Some studies have stated that the type and numbers of bacteria and viruses found in health-care solid waste are little different from that found in wastes generated from dwelling units, offices, factories and other institutions. Other researchers have given a completely opposite view and stated that health care facility wastes may be potentially dangerous to the environment due to their infectious content. (11)

Both hospitals and veterinary hospitals (for more specific breakdown by Standard Industrial Classification Code see §250.14 (b) of the regulations) are health care facilities that are considered to be generators of infectious waste for purposes of the regulation. EPA realizes that there are different problems associated with the infectious wastes from the treatment of people vs. animals and by no means does the Agency intend to imply that these two types of health care facilities generate the same types and amounts of waste or should treat or dispose of their wastes by the same methods. A discussion of each type of health care facility and sources of waste associated with them are given below.

Hospitals

Theoretically, the difference between the biological hazard of waste generated in hospitsls, with their population of "sick" people, and the waste generated by dwelling units and other buildings that are occupied basically by "well" people, lies in the waste's content. A proportion of the waste materials generated by hospitals in the treatment of patients has been exposed directly or indirectly to various pathogens in concentrated forms. From 2 to 8 percent of hospital wastes, for example, consists of such materials as: dressings from wounds, incisions, and burns; plaster casts; infectious laboratory samples; bacteriological cultures and media; pathological specimens; animal remains and biological specimens; body fluids and secretions; blood, urine, feces, and tissues; needles and syringes; disposable treatment devices made of plastic, metal, and glass; "sharps"; newborn, pediatric, and geriatric diapers; and various contaminated disposable containers. (12)

The leading generation points for these known infectious wastes are surgical suites, isolation wards for communicable diseases, clinical and research laboratories, research animal quarters, the autopsy suite and pathology laboratory, and the renal dialysis department. Another generation point is any care and treatment area or room for a known infectious case--inpatient, outpatient or emergency. As these wastes come from specific departments or sources, segregation is possible by handling all wastes from these particular areas as being infectious. The major problems in isolating possibly "infectious" wastes arise from the general patient care and treatment areas, both inpatient and outpatient, where large numbers of patients are being cared for by nursing personnel and diagnosis is often incomplete at the time. It is these areas that infection potential of most waste is unknown. So, at some point, there is a reasonable possibility that infectious wastes can be intermixed with other wastes.

Three surveys have been made which cover quite extensively hospital practices with regard to waste collection and disposal (Iglar and Bond, 1971; (13) Burchinal and Wallace, 1971;(14) Esco/Greenleaf, 1972 (15)). The main interest, however, has been in evaluating the overall waste collection and disposal systems, with infectious wastes being considered as only one aspect of the overall situation. This section is concerned with discussing the infectious wastes which are identified in the literature.

The composition of infectious wastes is well known. They include items from surgery such as dressings, contaminated disposable items, drapes, and human tissue (amputated limbs, tissues, organs, placentas); items from pathology and the laboratory such as tissues, chemicals, bacteriological cultures, urine, blood, and feces; animal remains and biological specimens; and general infected material from the wards such as gauze dressings and bandages, swabs, plaster casts, sputum cups, paper tissues soaked with nose and throat secretions, and wound drainage.

Some authors distinguish between "pathological" wastes and "hazardous" or "infectious" wastes (Litsky, et al., 1972). (16) They call "pathological" materials those from surgery, laboratories, etc., and "hazardous" waste everything else--everything from the hospital floor and everything that comes in contact with patients. Disposal systems in the hospital are often different for the two types of waste, but for transport and disposal away from the hospital these authors found that the two cannot be separated. The Esco/Greenleaf report (1972)(15) had this to say:

> "Early in the study we concluded that there is no practical way of segregating contaminated and uncontaminated waste in a hospital, and that, with few exceptions, contaminated and uncontaminated wastes are co-mingled together either on purpose or accidently so that by the time these materials reach the back door of the hospital for disposal ...there is no distinction...Therefore everything ...from a hospital floor must be considered to be contaminated and should be classified as waste."

This position is not uniformly held. Burchinal and Wallace (1971) (14) state that only 25 to 30 percent of the total waste generated in a hospital can be considered dangerous, and if this is kept apart from the remaining waste there is no need to treat the total waste as contaminated.

Surgery, autopsy, and the laboratories generate most of the segregated pathologic waste. The waste for the Los Angeles County - USC Medical Center is given in Table 8 (Esco/Greenleaf, 1972). (15)

An investigation by G.H. Reavely and P.G. Warwick of the University of Western Ontario (Anon, 1972c)(17) defined pathological wastes as "all substances which cannot be resterilized or reused originating within or brought into patient care, laboratory and autopsy areas." Patient care areas not only included those traditionally considered to be sources of infectious waste, but also ward areas, doctors' offices, outpatient clinics, and treatment rooms. Infectious waste averaged 43 percent of the total waste in the hospitals studied, and the general patient care areas generated almost three quarters of this infectious waste.

Table 8

Quantities of Pathological Wastes Generated Daily at LAC-USC Medical Center from Various Divisions

(Esco/Greenleaf, 1972)

Areas	Quantity of Waste (gal/day)
Lab Services (Basement)	30
Autopsy and Lab Areas (2nd Floor)	80
Laboratories (2nd Floor)	45
Pathology Lab (16th Floor)	30
Surgical Delivery (4th Floor)	2
Total	187 gallons/day

Using a density factor of 5.2 lbs./gal. based upon 70% moisture, a calculated production of 1000 lbs/day may be expected for the pathology incinerator.

A survey in California (Anon, 1972b) (18) concluded that it was possible to safely separate and collect infectious waste within a hospital, but this does result in increased costs of waste handling. With an average total waste per patient day of 10.25 lbs., the average infectious waste measured was only 0.38 lbs.

Investigations by Bond and Michaelson (1964)(19) on the effects of waste handling upon air and surface contamination give some indication of what types of contamination to expect. They found that soiled laundry handling had by far the most significant influence on increased airborne bacteria.

Further investigations have been carried out on the solid waste itself. Armstrong (1969) (20) looked at refuse chutes with respect to airborne bacteria. He found that placing the refuse in bags reduces the number of airborne bacteria generated, and that the possibility exists for the transmission of viable organisms to other parts of the hospital by way of the refuse chute.

Research at the University of West Virginia Medical Center (Burchinal and Wallace, 1971; (14) Wallace, et al., 1972; (21) Smith, 1970; (22) Trigg, 1971 (23)) revealed that pathogenic organisms can be present in hospital solid waste in significantly high concentrations, and especially so if an organic substrate is present. Coliform counts ranged from less than one per gram of refuse at some stations to as high as 8.6 per gram. Fecal streptococci ranged from less than 1 per gram to as high as 8.0 per gram; staphyloccocci from less than 2 per gram to 7.1 per gram; Candida albicans from less than 2 per gram to 3.8 per gram; Pseudomonas sp. from less than 2 per gram to 8.4 per gram, and spore counts from less than 1.5 to 3.9 per gram (Trigg, 1971).

Substantial numbers of organisms of human origin were found, which suggests the presence of virulent pathogenic bacteria and viruses living on the solid waste in undetected numbers. Bacillus organisms made up 80 to 90 percent of all microbes observed with staphloccocci and streptococci each composing between 5 and 10 percent of the population. Staphylococcus aureus was by far the most predominant pathogen detected in the waste. Spore forming organisms were not present in sufficient numbers to constitute a potential hazard if accepted methods of sterilization are followed. Nursing stations, such as the operating rooms, where pathological waste is separated from other waste, show much lower microbial concentrations in general refuse than other stations. The stations generating the refuse most highly contaminated with coliform bacteria are the intensive care units and pediatrics.

Virus survival studies indicate that almost all materials found in the hospital solid waste could be vehicles for transmission of viruses (Burchinal and Wallace, 1971; (14) Wallace, et al., 1972 (21)). Various types of waste were artificially contaminated with viruses to established recovery times and rates. Vaccinia, Polio 1, Coxsackie A-9, and Influenza PR-8 were the viral strains used for inoculation. Paper and cotton fabric both held active viruses for long periods of time--from 5 to 8 days in most cases. Virus titer decreased in most cases at a steady rate with increasing time, implying that the agent loses its viability upon incubation.

An air sampling program was carried out at the Los Angeles County-USC Medical Center (Esco/Greenleaf, 1972).(15) Results are given in Table 9 and substantiate the earlier findings of Bond and Michaelson that laundry handling does generate considerably greater aerosols than does trash handling.

Estimates of the total waste generated by hospitals vary widely, ranging from about 10 lbs/patient/day to as much as 40-50 lbs/patient/day (Litsky, *et al*., 1972; (16) Oviatt, 1969;(24) Wallace, *et al*., 1972; (21) Anon, 1972b(18); Small, 1971(25)). Tables 10 and 11 give a breakdown of the types of wastes generated and the disposal costs for seven California hospitals. The great variation is caused by the quantity of disposable items used. The trend has been toward greater use of disposables because of decreased danger of cross-infection and supposedly greater economy. It has now become evident that "disposables" are really merely "throw-aways"; and their actual *disposal* presents a large problem. Even the cost advantage is open to question; Table 12 indicates that disposables cost more to handle and dispose of than reusables.

Table 9

DRAFT

Summary of Air Sampling Data at LAC-USC Medical Center
(Esco/Greenleaf, 1972)

Station	Number of Observations	Mean Coliforms per cubic foot	No. of Samples wit Colonies too Numer ous to Count
Trash Chute Room			
Inside	99	14.1	2
Outside	96	8.8	0
Laundry Chute Room			
Inside	58	38.3	2
Outside	57	31.4	5
Sorting Area	54	71.0	7
Station Utility Room	55	5.0	0

Table 10

Breakdown of Daily Waste Production (Lbs/Day) By Types of Wastes (Esco/Greenleaf, 1972)

Type of Waste	LAC-USC Medical Center	Long Beach General Hospital	Harbor General Hospital	Ranchos Los Amigos Hospital	John Wesley Hospital	Olive View Hospital	Mira Loma Hospital
# of Beds	3000	428	715	1188	259	725	232
Sharps, Needles, Etc.	75	3	22	40	8	20	5
Path. & Surgical	1000	trace	156	4	115	6	trace
Soiled Linen (Reusable)	45,000	3,740	13,600	16,320	2,900	5,630	1,120
Rubbish	16,200	540	6,569	2,760	717	1,722	362
Reusable Patient Items	trace	trace	trace	trace	trace	trace	trace
Non-combustibles	1,500	75	465	725	80	250	80
Non-grindable (a) Garbage	1,800	150	660	875	160	475	110
Food Service Items (Reusable)	9,000	1,400	2,400	4,200	800	2,500	600
Radiological	trace	—	trace	trace	—	trace	—
Ash & Residue	trace	—	20	20	50	20	25
Animal Carcasses	25	—	220	20	10	23	—
Food Waste (Grindable)	2,600	330	950	1,100	210	1,860	150
Total Production	77,700	6,238	25,062	26,064	5,050	12,506	2,452

Table 10 (CONT.)

Daily Production Disposable	23,000	1,098	9,062	5,554	1,350	4,376	732
Pounds per bed patient	11.6	3.6	16.7	6.0	7.9	7.8	5.1
Pounds per capita. (b)	3.75	2.08	5.57	2.80	3.44	4.32	3.37
Daily Production Reusable	54,000	5,140	16,000	20,520	3,700	8,130	1,720
Pounds per bed patient	27.2	16.9	29.6	22.1	21.7	14.5	11.9
Pounds per capita (b)	8.75	9.74	9.73	10.20	9.41	8.08	7.93

(a) Predominantly garbage mixed with substantial quantities of paper, plastics, metal, etc.

(b) Per capita production based on equivalent 24-hour population.

Table 11

Annual, Daily, and Unit Operating Costs (Esco/Greenleaf, 1972)

	LAC-USC Medical Center	Long Beach General Hospital	Harbor General Hospital	Rancho Los Amigos Hospital	John Wesley Hospital	Olive View Hospital	Mira Loma Hospital
Quantity of Waste Produced							
Disposables (Tons/Day)	11.60	0.55	4.53	2.77	0.68	2.19	0.37
Reusables (Tons/Day)	27.25	2.57	8.00	10.26	1.85	4.06	0.86
Total Waste (Tons/Day)	38.85	3.12	12.53	13.03	2.53	6.25	1.23
Cost of System Operation							
Annual	$2,396,850	$223,600	$777,435	$656,340	$296,582	$750,585	$175,200
Daily	$ 6,566	$ 612	$ 2,130	$ 1,798	$ 813	$ 2,056	$ 480
Average Daily Cost per Ton							
Disposables	$ 305	$ 325	$ 327	$ 364	$ 664	$ 516	$ 551
Reusables	110	168	82	77	195	229	322
Total Wastes	170	197	170	168	321	329	390
Average Daily Cost/Bed Patient [Calculated based on total number of patients not total number of beds].							
Disposables	$ 1.76	$ 0.58	$ 2.73	$ 1.09	$ 2.65	$ 2.02	$ 1.42
Reusables	1.49	1.44	1.21	.85	2.13	1.65	1.91
Total Wastes	3.25	2.02	3.94	1.94	4.78	3.67	3.33

Table 12

Cost Comparison of Disposable and Reusable Wastes at LAC-USC Medical Center
(Esco/Greenleaf, 1972)

Daily Costs of Handling and Disposal

Type of Waste	Ave. Wt. lbs/day	Labor	Bldg. & Equip.	Other (a)	Total	Ave. Cost	
						Per ton	Per Bed
Disposables							
Rubbish	16,200	$2,235	$104	$ 85	$2,424	$300	$1.22
Other	7,000	1,027	40	60	1,127	322	.56
Total	23,200	$3,262	$144	$145	$3,551	$305	$1.78
Reusables							
Soiled Linen	45,000	$1,255	$ 45	—	$1,300	$ 57	$0.65
Food Service Items	9,000	1,403	—	—	1,403	312	.70
Other	trace	312	—	—	312	—	.15
Total	54,500	$2,970	$ 45	—	$3,015	$110	$1.50
Total All Materials	77,700	$6,232	$189	$145	$6,566	$170	$3.28

(a) Miscellaneous expendable supplies and dumping fees.

Disposable items are found in all the areas of the hospital, and have special application in burn therapy, aseptic techniques, and isolation cases. Typical items are found in Table 13. They are combinations of materials such as paper, plastic, rayon, acrylic, cellulose, nylon, glass and metal. The plastic content is much higher than the 2-3 percent found in municipal solid waste; one study of infectious waste found it to be 11.42 percent hard plastic and 7.09 percent soft plastic (Anon, 1972b).(18) Expenditures have risen from $30 million in 1966 to $126 million in 1970, and may rise to an estimated $900 million in 1978 (Fahlberg, 1973).(26) Further estimates say that a hospital can double its waste output by completely switching to disposable linen (Salkowski, 1970).(27) Disposables add two problems to the waste treatment process; first they increase the volume so that disposal systems are taxed and second the plastic components are hard to degrade. Also, it may be that some plasticizers are toxic. The John Hopkins School of Hygiene and Public Health in Baltimore has found that plasticizers in blood bags leach into the stored blood and go on to lodge in lungs, spleen, liver, and abdominal fat. Tests of embryonic heart cell cultures revealed that the cells died when plastic tubing was substituted for rubber (Anon, 1971b). (28)

When a simple a change as supplying paper towels to each patient's room was made at the Baylor University Medical

Table 13

Common Disposable Items Used in the Hospital

Catheters and catheterization trays

Cutting Blades

Eating utensils

Emesis basins

Enema administration bags and buckets

Examination gloves

Exchange transfusion trays with tubing and fittings

Foley catheter trays

Hypodermic syringes with and without attached needles

Hypodermic needles

Hypodermic syringes pre-filled with medication

Irrigation trays

Lumbar puncture trays

Manometer trays

OB and surgical packs

Oxygen canopies

Petri dishes

Prefilled nursers

Prepared enemas

Sheets and pillowcases

Spinal anethesia trays

Surgeons gloves

Surgical prep trays

Suture removal kits

Venous pressure trays

Center, it was found an additional wastebasket was then required. The maintenance cost from plugged toilets increased, and the labor charge for emptying and washing wastebaskets increased by 30 percent, but the number of cloth towels used did not decrease (Paul, 1964).(29) The pure bulk of the disposables presents the problem that most authors comment on, but other hazards are also present. Discarded needles and cutting edges remain a hazard to collection personnel. Scavenging of the dumping areas for useable items and play items for children show that spread of infectious disease is a real hazard in the disposal of disposables (Walter, 1964; (30) Mattson, 1974 (31)). Disease organisms can also be introducted to a landfill in great quantities via disposable linens and diapers (Ostertag and Junghaus, 1965; (32) Peterson, 1974 (33)).

Some indication of the numbers of disposable hypodermic needles used by individual hospitals can be obtained from the literature. Michaelson and Vesley (1966)(34) found from 14,000 to 833,000 used annually at various hospitals in 1966, and Baker (1971)(35) found over 550,000 used annually in 1968. There are proper ways to collect and destroy these items, such as collecting them at the individual nursing stations and returning them to central storage to be crushed and broken into fragments, then incinerated. They can also be collected in special boxes and sent directly to the incinerator, or collected at the nursing stations and sent to central service to be autoclaved and melted into one mass (Paul, 1964). (29) Some hospitals have even tried replacing the needles on a one for one basis as they are used, then destroying the old ones (Deschambeau, 1967).(36) Even though the users are aware of the need to destroy the waste syringes and needles, many still escape unscathed. Profit oriented hospital workers have been known to extract these from the daily waste and sell them to street drug users (Hewer, 1971).(37) Even at the final landfill site, these needles can be reclaimed for drug users and children who find them to be satisfactory squirt guns (Healy, 1965).(38)

Based on the above discussion, the Agency concluded that it is necessary to regulate only certain sources of infectious waste within hospitals, rather than all waste from these facilities. Further, the Agency concluded that it is unnecessary to regulate waste materials from these sources which have been properly treated by the hospital to render them non-infectious (see §250 Subpart A Regulations, Appendix VII, Infectious Waste Treatment Specifications.)

The following departments of hospitals are subject to Subtitle C regulation:

Obstetrics department including patients' rooms
Emergency departments
Surgery department including patients' rooms
Morgue
Pathology department
Autopsy department
Isolation rooms
Laboratories
Intensive Care Unit
Pediatrics department

Veterinary Hospitals

While veterinary hospitals have some of the waste disposal problems which hospitals caring for people have, these problems are mainly confined to disposing of dead animals, animal waste, and waste generated during treatment of animals. Animal waste includes waste generated from animal care or use, including excretions, secretions, tissue, remains, and any inedible by-products of animal processing for food and fiber production.

It has been pointed out to the Agency that the majority of diseases that could be transmitted through improper disposal of veterinary hospital waste are primarily ones that are transmitted only from animal to animal. It is true that several hundred diseases are transmitted from animal to animal, but more than 150 zoonotic diseases are transmitted between animals and man.

Decker and Steele (38a) report the human health problems that are created by pathogenic zoonoses. Some of the most significiant bacterial zoonoses are salmonellosis, staphlococcal and streptococcal infectious, tetanus, tuberculosis, brucellosis, leptospirosis, and colibacillosis. Animal wastes also play a significant role in the distribution of fungal diseases by providing nutrients for the survival and growth of fungi in man's environment.

Q fever, a rickettsial disease, is transmitted to man primarily through air laden with dust containing animal wastes. It is largely an occupational disease of cattlemen, slaughterhouse workers, and woolsorters, but may also attack those residing adjacent to feedlots and stockyards. A troublesome parasitic disease transmitted through animal wastes is trichinosis which persists even though the practice of feeding swine raw garbage has been greatly reduced in recent years.

Less is known regarding the role of animal wastes in the direct transmission of viral diseases than in bacterial diseases. However, the importance of animal wastes in the reproduction of insect vectors of many diseases is well documented.

Anthrax

Anthrax is one of the oldest diseases identified with animals that is transmissible to man. Anthrax has been present in the United States for at least the last 100 years. The disease is primarily an occupational hazard of industrial workers who process hides, hair (especially from goats), bone and bone products, and wool, and of veterinarians and agricultural workers who handle infected animals. (39)

Infection of the skin is by contact with tissues of animals (cattle, sheep, goats, horses, pigs, and others) dying of the disease; or contaminated hair, wool, hides, and soil associated with infected animals. Inhalation anthrax results from inhalation of anthrax spores. Gastrointestinal anthrax arises from ingestion of contaminated undercooked meat. Anthrax spreads among herbivorous animals through contaminated soil and feed and among omnivorous animals through contaminated meat, bone meal or other feeds. Biting flies and other insects are suspected of serving as vectors. Vultures have spread the organism from one area to another. The spores of *Bacillus anthracis*, the infectious agent, which resist environmental factors and disinfection, remain viable in contaminated areas for many years after the source-animal infection has terminated. (39)

Initial symptoms of inhalation anthrax are mild and non-specific, resembling common upper respiratory infection; acute symptoms of respiratory distress, fever and shock follow in from 3 to 5 days, with death shortly thereafter.

Gastrointestinal anthrax is more difficult to recognize, except that it tends to occur in explosive outbreaks; abdominal distress is followed by fever, signs of septicemia, and death in the typical case.

Untreated cutaneous anthrax has a fatality rate of from 5-20%, but with effective antibody therapy, few deaths occur. (39)

Salmonellosis

Although this disease is discussed in the section on sewage sludge, the important role that animals play in the transmission of the disease shall be stressed here.

Animal excreta and inedible by-products of food processing, such as viscera, bones, and feathers are vehicles that carry salmonella organisms from their animal hosts to man.(40) Direct contact with such wastes constitutes an occupational hazard for livestock producers, slaughterhouse and rendering plant workers; contamination of edible food products with feces provides a means of carrying the organism to the consumer, to the home, or to the institutional environment.

Animal wastes are also a vital factor in perpetuating and extending the prevalence of animal hosts of the Salmonellae. (41) Feeding of animal feces to poultry, swine, beef, and dairy cattle is one means of increasing the incidence of animal salmonella hosts, as is the use of contaminated animal protein supplements in animal feeds.

In 1965 a waterborne outbreak in southern California affected some 16,000 people. How the water supply of the city of Riverside became contamined is unknown, but *Salmonella typhimurium* (Phage II), the cause of the outbreak, is widely disseminated in animals not only in California but throughout the world. There has been speculation that contamination could have originated in feedlots where cattle were passing *Salmonella typhimurium* hundreds of miles away, and due to seepage along earthquake faults, the bacteria appeared in the water supply. (38)

Tuberculosis

Tuberculosis must still be considered as an important disease related to animal wastes. While bovine tuberculosis caused by *Mycobacterium bovis* has been effectively controlled in this country, it is occasionaly found in some wild animals, as well as in food animals and in pets.

Mycobacterium tuberculosis, the human type of tubercule bacillus, is capable of infecting cattle swine, and household pets.

Mycobacterium avium, the etiologic agent of tuberculosis in gallinaceous birds, is capable of producing tuberculosis in swine and of infecting cattle to such an extent that reactions are produced in routine tuberculin testing of cattle.

The bovine tubercle bacillus is transmitted to man through respiratory secretions, feces, and milk. In those few cases where infection of man with the bovine tubercle bacillus is known, there usually is an occupational contact with cattle. (38)

Brucellosis

Brucellosis is commonly an occupational disease of those with close contact with cattle and swine and their viscera and excreta. The disease in man and animals is caused by any one of three species of *Brucella*.

Brucella abortus is predominantly of bovine origin, Brucella suis of swine origin, and Brucella melitensis primarily infects goats. Cows may become infected with Brucella suis or Brucella melitensis as well as Brucella abortus. Swine may become infected with all three species; however, they are most susceptible to Brucella suis. Many outbreaks of brucellosis have been traced to contaminated water courses from meat-processing plants, rendering plants, and contaminated farms. (38)

The disease is systemic, with acute or insidious onset, characterized by continued, intermittent or irregular fever of variable duration, headache, weakness, profuse sweating, chills, or chilliness, arthralgia, depression, and generalized aching. Non-purulent meningitis and pneumonitis may occur. The disease may last for several days, many months, or occassionally several years. Orchitis and vertebral osteonmyelitis are uncommon but characteristic features. Recovery is usual but disability is often pronounced. The fatality rate is 2% or less; higher for Brucella melitensis infections than for other species. Clinical diagnosis is often difficult and uncertain. Death is rare in persons without complications. (39)

Leptospirosis

Leptospirosis is a spirochetal disease of large proportions and is world-wide in distribution. A number of animal species host the *leptospira*, including the domestic food-producing species. Cattle and swine are the principal domestic animals involved--leptospirosis occurs in epizootic form in stables and feedlot herds. Dogs and rodents are frequently infected.

Leptospirae are transmitted from the animal host to man through a number of routes. Documented sources of human infection are rice fields, swimming "holes", sewers, and a number of occupations in which exposure to infected animals is by direct contact. (38)

The disease in man shows a wide range of symptoms and severity, depending on the species of leptospira involved, exposure, and the health of the individual. It presents symptoms similar to influenza, enteric viral infections, infectious gastroenteritis, and a number of other diseases. Fatality is low, but increases with advancing age and may reach 20% or more in patients with jaundice and kidney damage. (39)

Tularemia

The reservoir for Tularemia is normally wild animals, but is occasionally found in sheep. Mode of transmission is by inoculation of the skin, conjunctival sac or anal mucosa with blood or tissue while handling infected animals, as in skinning, dressing, or performing necropsies; or by fluids from infected flies, ticks, or other animals, or through the bite if arthropods including a species of deer fly. The disease is characterized by sudden and dramatic onset of chills and fever. Fatality in untreated cases is about 5%; with treatment, negligible. (39)

Although the above discussion of disease transmitted to man from animal has centered on occupational hazard data, the same types of wastes are generated from certain departments of veterinary hospitals. Again, as with hospitals, the Agency has concluded that only a portion of the total waste load of veterinary hospitals is a source of infectious waste (unless properly treated prior to disposal to render non-infectious).

For purposes of identifying sources of infectious waste, the following departments of veterinary hospitals are subject to Subtitle C regulation:

Emergency department
Surgery department including patients' room
Morgue
Pathology department
Autopsy department
Isolation rooms
Laboratories
Intensive care unit

NOTE: The Agency realizes that the names of the above departments are normally applied to hospitals for humans; the departments of veterinary hospitals that are functionally equivalent would be applicable.

3.11 Rationale for Regulation of Laboratory Waste

Data are generally not available that can be used to show evidence of disease associated with laboratory waste. In a recently published study at the University of Texas (Pike, 1975) (42), some waste/disease data can be extracted from the 50-year data base of published and unpublished cases of laboratory-associated infections.

As shown in the reproduced table (Table 7), 46 cases of laboratory-acquired infections related to the (waste) source of discarded glassware are shown. Of these cases, 34 were related to bacteria, 10 related to viruses, and 2 to rickettsiae. Of the total number of reported laboratory-associated infections studied, the 46 associated with discarded glassware represent about 1% of the total.

The Center for Disease Control has determined that certain microorganisms are of potential hazard to human health and the environment, as published in the "Classification of Etiologic Agents on the Basis of Hazard." Since it has been determined by HEW that classes 2 through 5 are of potential hazard, then any laboratory dealing with these agents would be generating a potentially hazardous, infectious waste. Given that most hospitals and laboratories know which organisms are used in their work, the list is appended to the regulation to indicate the type of laboratory which would be included by the specified SIC codes. It must be recognized however, that many times in diagnostic work the organisms involved are unknown. By regulating laboratories as the "source" of infectious waste, the unknown presence of pathogenic organisms can be controlled. Thus, the CDC list is used as a basis for including laboratories as a source of infectious waste, but the list cannot be used alone to define this source, due to the nature of the waste from diagnostic labs.

TABLE 7 - Distribution of Cases According to Proved or Probable Source of Infection

	Agents							
Sources	Bacteria	Viruses	Rickettsiae	Fungi	Chla-mdiae	Parasites	Unspec-ified	Total
Accident	378	174	45	33	14	38	21	703
Animal or ectoparasite	149	249	66	151	32	11	1	659
Clinical specimen	90	175	2	1	0	19	0	287
Discarded glassware	34	10	2	0	0	0	0	46
Human autopsy	56	9	4	0	0	1	5	75
Intentional Infection	14	1	0	0	0	4	0	19
Aerosol	101	92	217	88	22	2	0	522
Worked with the agent	381	213	100	62	43	28	0	827
Other	7	1	7	0	1	0	0	16
Unknown or not indicated	459	125	130	18	16	12	7	767
Total	1669	1049	573	353	128	115	34	3921

3.12 Rationale for Regulation of Unstabilized Sewage Treatment Plant Sludge

The Agency has decided to regulate "domestic" or "municipal" sewage sludge form publicly owned treatment works under the authority of Section 405 of the Clean Water Act, supplemented by Section 4004 of RCRA. Unstabilized sewage sludge from industrial or other sources is considered to be a hazardous waste subject to regulation under Subtitle C of RCRA. Thus the following discussion applies to sewage sludges from industrial and other sources, which in many cases are similar or identical in character to domestic or municipal sludges.

The fact that pathogens do survive in sewage sludge has been addressed by EPA in the November 1977 Federal Register notice entitled "Municipal Sludge Management: Environmental Factors; Technical Bulletin." (43) In this publication, EPA recommends that sewage sludge be "stabilized" before landspreading "to reduce public health hazards and to prevent nuisance odor conditions." Stabilization of sewage sludge is defines as chemical, physical, thermal, or biological treatment processes that result in the significant reduction of odors, volatile organics, and pathogenic organics. EPA, in the same publication, recognizes that "although these conditions can reduce the number of influent fecal coliforms by 97 percent or more, the remaining levels of microorganisms may still have public health significance". And, further, that "under certain conditions. . .it may be necessary to achieve additional bacterial, parasite, and/or virus reduction beyond that attained by stabilization.'

In this bulletin general requirements for land application of sludges are given. Reference is made to "Process Design Manual for Sludge Treatment and Disposal" (EPA 625/1-74-006; October 1974) which specifies in more detail the techniques for sludge stabilization.

The bulk of the information presented in this section of the background document is identical to that presented in the background document for §257.4-5 (Land Criteria) to be used for Section 4004 of RCRA. (45) Section 4004 regulations will require sewage treatment plant sludge to be "stabilized" to "reduce public health hazards."

Pathogenic organisms occuring in sewage sludge cover a wide variety of bacteria, viruses and intestinal parasites. Their individual presence, as well as their numbers, will vary considerably from community to community depending upon rates of disease in the contributing population. (46) Routes of infection to humans and animals from sewage sludge may be through direct contact with contaminated environments or through the ingestion of contaminated food and water.

Bacteria

Among the bacteria that are commonly found in sewage sludge, is the group referred to as the "enteric bacilli" that naturally inhabit the gastronintestinal tract of humans. In their virulence for humans, the enteric baccilli fall into three general categories: pseudomonas species, salmonella species, and shigella species.

Pseudomonas

The pseudomonas species include the proteus organisms, Pseudomonas aeruginosa, and Alcaligenes faecalis. These common inhabitants of the normal human gastrointestinal tract are ordinarily non-pathogenic, causing disease (most often of the urinary tract) only under special circumstances.

Salmonella

The genus Salmonella contains a wide variety of highly invasive "species" pathogenic for humans or animals, and usually for both. Largely as the result of systematic studies, over 700 Salmonella species have been identified on the basis of specific antigens. Three distinguishable forms of salmonellosis occur in humans: enteric fevers, septicemias, and acute gastroenteritis.

The prototype of enteric fever is caused by Salmonella typhosa. The organism is usally acquired by ingestion of contaminated food or water, and the focus of occurence in the United States is in the South. There were 375 cases of typhoid fever reported in the U.S. for the year 1976.(47)

The second form of salmonellosis is Salmonella septicemia, which is characterized by high, remittent fever and bacteremia, ordinarily without apparent involvement of the gastrointestinal tract. The third form, gastroenteritis, is a disease confined primarily to the gastrointestinal tract, and in most cases is caused by the Salmonella sp. typhimurium.

Shigella

The third category of enteric bacteria is the Shigella genus. The shigella cause in humans a disabling disease known as bacillary dysentery. This is an acute infection of the large intestines, resulting in diarrhea, which, if sufficiently severe, may be accompanied by bleeding from the colon. All known species of the genus Shigella are pathogenic for humans, with the following being the most common: S. dysenteriae, S. flexneri, and S. sonnei.

None of the enteric bacilli form spores. Spores are resistant bodies produced by large number of bacterial species that enable them to withstand unfavorable environmental conditions such as heat, cold, desiccation and chemicals. Since enteric bacilli are not spore formers, their survival span outside of their normal environment (human intestinal tract) is usually measured in days or months, compared to years for spore forming bacteria. Most sludge stabilization processes would create an unfavorable environment for enteric bacilli to survive.

A pathogenic bacterium frequently found in sewage sludge, although not an enteric organism, is the tubercle bacillus Mycobacterium tuberculosis. This organism is responsible for nearly all cases of pulmonary tuberculosis. Tubercle bacilli are very hardy organisms, and can withstand fairly extreme environmental conditions.

Viruses

The second group of pathogenic organisms found in sewage sludge are the enteric viruses. Viruses present certain differences from bacteria and possess many characteristics peculiar to their own group. Biologically, the most important difference between viruses and bacteria is that viruses must invade the living tissue cells or bacteria cells to multiply within them, whereas the bacteria do not invade the cells of their host.

More than 70 serologically distinct human enteric viruses can occur in sewage sludge. (48) The major pathogenic enteric virus groups are the Polio viruses, Coxsackie viruses, Echoviruses and the Hepatitis virus.

Poliomyelitis, caused by the poliovirus, is an acute systemic infection which, in its clinically recognizable form appears as an involvement of the central nervous system and often results in a variable degree of permanent paralysis. The escape of the virus from the body of the infected person is in respiratory tract secretions and in the feces.

Coxsackie viruses ara responsible for common enteric infections and a variety of illnesses, including several clinically distinct ones in humans.

Echoviruses comprise a group of biologic agents brought together chiefly because they infect the human intestinal tract. Certain species are known to cause aseptic meningitis, febrile illnesses and diarrheal diseases in infants and children.

Infectious hepatitis is an acute infectious disease that causes fever, nausea, abdominal discomfort, followed by jaundice. It is caused by a resistant virus. The *Hepatitis virus* is shed from the body through the feces, and fecal-oral spread is probably the most common method of transmission.

Parasites

The third group of pathogenic organisms found in waste water treatment sludges are the intestinal parasites. Those parasites of concern to humans can be subdivided into two categories: (1) *Protozoa*, and (2) *Helminths*. Subgroups of the *Protozoa* group include amoebas, flagellates, and ciliates. Subgroups of the *Helminths* include trematodes and nematodes.

Protozoa

At least five species of amoebae live in the intestinal tract of humans, with *Entamoeba histolytica* being the only proven pathogen. Infection with *E. histolytica* may produce chronic diarrhea, amoebic hepatitis, abscess of the liver, brain, lung, and ulceration of the skin. Amoebae have two stages in their life cycles, a mobile form and a cyst form. The cysts are infective upon passage from the body, and are survive in a moist and cool environment. *Giardia lamblia*, another protozoan, is also found in sewage sludge. Like the amoeba, *G. lamblia* is a parasite of the human intestinal tract and is responsible for certain conditions such as diarrhea or symptoms referable to the gall bladder.

Balantidium coli is the only ciliate human parasite and is the largest of human protozoan parasites. It invades a tissue and produces intestinal pathology similar to that of *E*. *histolytica*.

Helminths

Helminths are commonly referred to as worms. In a more restricted sense the name worm, or preferably helminth, is applied to a few phyla of animals, all of which superficially resemble one another in being "wormlike," though in life and structure they are widely different. *Ascaris lumbricoides* is the longest-known human parasite in this group. It was not until early in the present century that Ascaris was recognized as being as injurious and sometimes dangerous parasite.

Ascaris lumbricoides is a large nematode; the females commonly reach a length of 8 to 14 inches. The adult normally lives in the small human intestine, where it commonly bites the mucous membrances to extract tissue juices. Ascaris produce a tremendous number of eggs (ova) which are passed out of the body in the feces. Infection ordinarily results from swallowing the embryonated eggs, which are in most cases conveyed to the mouth by food or water. In heavy infections the migration of the larvae through the lungs causes hemorrhaging and sets up a severe pneumonia which may be fatal. The ova of the Ascaris are extremely durable, and are capable of withstanding severe environmental conditions.

Other *Helminths* encountered in sewage sludge are the tapeworms or *Cestoidea*. Although 25 or 30 different species of tapeworms have been recorded in man, only 4 adult species are to all common. These are *Dibothriocephalus latus*, *Taenia* *solium*, *T. saginatta*, and *Hymenolepis nana*. With the exception of the species of *Hymenolepis*, infection with the common human species results from eating raw or imperfectly cooked beef, pork, or fish in which the larvae have developed. *Hymenolepis sp*. on the other hand, need no intermediate host. It is able to complete its entire life cycle in a single host; thus, when eggs are ingested by man, the larvae migrate into the lumen of the intestine.

Numerous studies report that pathogenic organisms present in sludge are either killed or greatly reduced in number when exposed to various stabilization methods used.

The specific number of an organism necessary for the establishment of the potential for disease is related to various factors; etiologic agent, susceptibility of host etc. However, there is evidence that with many pathogens this dose may be rather high, in particular the enteric pathogens. DuPont et. al (49) reported that approximately 10^5 *Salmonella* cells (including S typhi) are required to cause a disease. This would tend to support the premise that by reducing the number of pathogenic organisms in sludge, the public health hazards associated with its use would be greatly minimized.

A review of the literature (7) has shown that there is a paucity of epidemiological data linking disease transmission of humans and animals directly to the landspreading of wastewater treatment sludges. The data that do exist, indicate that the transmission of enteric disease or parasitic infestation were related to the use of raw or unstabilized sludges on cropland. Sepp (50) in his literature review on the landspreading of wastewater sludge, lists numerous reports of infection both to humans and animals believed to be caused by ingestion of raw vegetables fertilized by raw sludges. In specific cases, Kreuz (51) and Kroger (52) reported disease outbreaks caused by _Salmonella_ species on lettuce grown on soil fertilized by raw sludge. Such evidence indicates that there is a public health risk associated with the landspreading of _unstabilized_ sludges.

Data linking disease transmission to humans and animals from the landspreading of stabilized sludges is virtually nonexistent. This lack of data can possibly be attributed to the fact that most individuals can tolerate the number of pathogenic organisms that survive the sludge stabilization process, or the ingestion of these organisms result only in sporadic cases of infection, of which the source is difficult to trace. Based on the knowledge of the human immune system, the former is a more plausible assumption. Work by Dupont et al (49) tends to support the former possibility, since their studies indicated that with many pathogens the infective dose may be rather high, in particular the enteric pathogens.

The stabilization process will reduce the pathogen population in sludge; the level of reduction will vary with the process used and numerous other variables, e.g., time, temperature, pH etc. Since available epidemiological evidence links disease transmission to the landspreading of unstabilized sludge and not stabilized sludge, it is evident that there is a correlation between the concentration of pathogens in the sludge and disease transmisssion.

Wastewater sludge stabilization is normally accomplished by anaerobic and aerobic digestion, and lime treatment. Lesser used methods include heat treatment, ponding and long time storage, chlorination, and composting. The stabilization of sludge by thermal irradiation is being addressed, but at this time the process is still in the experimental state.

As previously mentioned, the extent to which pathogenic organisms are reduced is related to the stabilization process used as well as other variables. Not all stabilization processes affect pathogenic organisms in the same manner, therefore, some processes are more effective in reducing the pathogen population than others. Also the levels of stabilization within a particular process will vary as to their effectiveness in reducing pathogenic organism numbers, e.g., anaerobic digestion of sludge for a two week period in the thermophilic range (125 F), is more effective in reducing pathogens than sludge digested anaerobically for two weeks in the mesophilic range (95 F).

The following is some of the information encountered relative to the effectiveness of various sludge stabilization processes in reducing pathogenic organisms. Table 3 summarizes these findings.

During anaerobic stabilization, the sludge temperature may reach 149 F by microbial action. However, the normal range for essentially all digesters in the United States is between 80 F to 100 F. (44) Although conditions in the digester are unfavorable for multiplication of most pathogenic organisms, they are not lethal and the principal bactericidal effect appears to be related to natural die-off with time. (44)

Kabler (53) reported that anaerobic digestion was comparatively ineffective in the inactivation of parasitic ova. Viable Ascaris eggs have been recovered following anaerobic digestion for as long as three (54) and six (55) months. An analysis of raw sludge from two community wastewater treatment plants revealed the presence of helminth ova and salmonella species. The same sludge after being stabilized by anaerobic digestion tested negative for both organisms.(56) Rudolfs et al. reported that after 6 months exposure to the anaerobic digestion process at 75 to 85 F, 46 percent of the ascarid eggs appeared normal. Other studies (54,57) reported that anaerobic digestion with different retention times removes the eggs of *A. lumbricoides* 0 to 45 percent.

Two groups (58,59) observed that there was 90 and 69 percent diminution of tubercle bacilli, while two others (60,61) noted "survival" of *M. tuberculosis* after anaerobic digestion.

McKinney et. al(62) found in their studies that approximately 93 percent of *S. typhosa* were removed after being exposed to anaerobic digestion process for 20 days. Kenner (63) reported that sludge treated by anaerobic digestion has been shown to contain *Salmonella* and *Pseudomonas* organisms.

Cram (54) reported from his studies, that activated sludge treatment does not affect the viability of *E. histolytica* cysts or ascarid eggs. Aeration in the activated sludge process for 5 months showed no effect on ascarid eggs except a slow reduction in numbers (64), Kabler (53) reported that studies indicate that activated sludge reduced *S. typhosa* and strains of bacilli 91 to 99 percent.

Table 3 Removal of Pathogens by Sewage Treatment Processes (53)
(Percent)

	Trickling Filter	Activated Sludge	Anaerobic Digestion	Chlorination	Stabiliza tion Ponc
Enteric Bacteria					
Total Counts	70-95	70-99	-	96-99	-
Coliform	82-97	91-93	-	99-99+	59-99+
Fecal Strep	84-94	-	-	-	-
Typhoid group	84-99+	Present: 95-99.2	Not found: 25-092.4	98-99	41/ml;N.D.
Shigella	-	97-98	-	-	-
Cholera	-	Not found	-	-	-
M. Tuberculosis					
M. Tuberculosis	Survive; 66-99	Survive; 88	Survive; 69-90	Survive; 99+	-
Entero viruses					
Polio	-	Survive	-	99	-
Coxsackie	Reduced; 60	Survive	Survive	-	-
ECHO	-	-	-	-	-
Infectious hepatitis	-	-	-	-	Survive or inactivated
Parasites					
Tapeworm Ova	18-26	Not removed	97	No effect	-
E. histolytica cysts	88-99.9	No reduction	Removed	-	-
Ascaros lumbricolides ova	-	-	45; reduced	-	
Taenia saginata	62-70	Little effect;	Very slow		

Enteric virus inactivation during the treatment of wastewater by the activated sludge process has been reported extensively in the literature. (65-70) Carlson (71) et al reported that after 6 months of aeration, polioviruses were removed or inactivated to a point at which infectiousness for mice was greatly reduced. Sproul (72) reported that virus removal of 90 percent or more has been obtained in a number of studies with activated sludge process. Kelly et al (73) reported that Coxsackie virus survived activated sludge treatment.

Table 4

Removal of viruses by bench scale activated sludge units

	Coxsackie virus A9		Poliovirus 1	
Test No.	Volatile solids (mg/l)	Virus Inactivated (Percent)	Volatile solids (mg/l)	Virus Inactivated (Percent)
1	600	98.8	200	79
2	650	96.1	400	88
3	1,000	99.2	600	90
4.	1,100	99.1	600	91
5	1,500	97.4	1,200	92
6	1,500	99.4	1,200	91
7			4,000	94

Bacterial inhibition from caustic conditions has long been known.(74) Studies have shown that Salmonella typhosa did survive in concentrations in the range of pH 11.01-11.50 longer than two hours, while Shigella dysenteriae was destroyed rapidly in all pH range studies; pH 11.01-11.50 produced 100% kill in 75 minutes. (75) However, the effectiveness of lime treatment on parasitic ova and viruses has not been demonstrated.

Destruction of pathogenic organisms in sludge or in sludge-refuse mixtures by composting has been reported extensively in the literature. (76-83) Table 5 indicates that 60 C (140 F) for one hour appears to kill all pathogens, with possible exception of Tubercle bacillus. (84) M. tuberculosis was shown to be destroyed within two weeks at temperature 60 C (140 F) or above. (55)

Table 5*

Time-Temperatures Required for Organism Destruction (84)

Organism	Destruction Time-Temperature		Destruction Time-Temperature	
	Temp (.F)	Time (min)	Temp (.F)	Time (min)
Salmonella typhosa	131-140	30	140	20
Salmonella sp.	131	60	140	15-20
Shigella sp.	131	60	—	—
Ent. histolytica cysts	113	few	131	few seconds
Taenia saginata	131	few	—	—
Mycobacterium tuberculosis var. hominis	151	15-20	152.6	momentary
Necator americanus	113	50	—	—
Ascaris lumbricoides eggs	122	60	—	—

* Adapted from Gotass

Long-term storage of sludge has been suggested as one of the simplest methods of reducing pathogenic organism numbers (85). Hinesly (86) reported that after storage of sludge for 30 days, fecal coliforms were reduced by 99.9 percent. However, Dotson (87) thought that parasites would probably persist much longer.

Heat treatment is a well known method of destroying pathogenic organisms. Three methods that have been applied to sludge treatment are low pressure oxidation, heat drying and pasteurization. During the low pressure oxidation (LPO) process, the sludge temperature is elevated to between 350 and 400 F, pressure is raised to 180 to 210 psi, and the retention time is between 20 and 30 minutes. The process kills all pathogenic organisms due to the high temperature achieved and the retention time. Over 26 U.S. cities are currently using the LPO process.

Heat drying of sludge is presently being carried out in a number of U.S. cities. However, the numbers are declining because of cost of fuel necessary for the drying process, and also because the market for heat dried sludge did not develop as hoped. The temperature achieved during the heat drying process kills most bacteria.

Pasteurization is a process where the sludge is heated to a specific temperature for a period of time that will destroy pathogenic organisms. In most cases this is accomplished by the use of steam. Currently, pasteurization is used only in Europe.

While the technical literature presents some conflicting data as to the degree that pathogenic organisms are reduced by various sludge stabilization methods, it does generally indicate that the stabilization process will reduce most pathogenic organisms significantly. This reduction, in turn minimizes the public health risks associated with the landspreading of stabilized sludges.

To survive and remain virulent, pathogenic organisms usually depend on the favorable conditions of a host. When an organism encounters a situation in which it cannot function normally, growth stops and the organism dies. Numerous environmental conditions may affect the organism after it leaves the natural host. Although organic matter in the sludge acts as a protective agent, organisms are stressed by waste treatment and encounter unfavorable moisture conditions, pH, temperature, sunlight, and nutrient levels when applied to land. Toxic substances in the sludge, soil antibiotics, and antagonistic organisms may also present obstacles to pathogen survival.

In soils receiving sewage sludge, most pathogens will disappear or be reduced to low numbers in two to three months. Although some pathogens have long survival time in soil (Table 6), most do not survive long on plant surfaces. When long survival times have been reported, initial inoculation levels were high, most pathogens were subsequently detected in low numbers, and no indication was given of the actual disease potential. (88)

Table 6 contains part of the data extracted by Dunlop (89) from his literature review pertaining to the survival of pathogenic organisms in soil, water and crops. Except for *Ascaris* ova, the table shows that most pathogenic organisms die off within one year. The two studies reporting *Ascaris* ova living 2-7 years were both conducted in Europe. Muller (90) reported in Germany that *Ascaris* ova survived up to 7 years

Table 6

Survival times of Pathogenic Microorganisms in various media (89)*

Organisms	Medium	Type of Application*	Survival time
Ascaris Ova	Soil	Not stated	2-5 years
	Soil	Sewage	Up to 7 years
	Plants and Fruits	AC	1 month
Endamoeba Histolytics cysts	Soil	AC	8 days
	Tomatoes	AC	18-42 hours
	Lettuce	AC	18 hours
Enteroviruses	Roots of bean plants	AC	At least 4 days
	Soil	AC	12 days
	Tomato & pea roots	AC	4-6 days
Salmonella	Strawberries	AC	6 hours
	Soil	AC	74 days
	Soil	AC	70 days
	Soil	AC	At least 4 days
	Pea plant stems	AC	14 days
	Radish plant stems	AC	4 days
	Soil	AC	Up to 20 days
	Lettuce & endive	AC	1-3 days
	Soil	AC	2-110 days
	Soil	AC	Several months
	Lettuce	Infected feces	18 days
	Radishes	Infected feces	53 days
	Soil	Infected feces	74 days
Salmonella, other than typhi	Soil	AC	15-70 days
	Vegetables	AC	2-7 weeks
	Tomatoes	AC	Less than 7 days
	Soil	Sprinkled with domestic sewage	40 days
	Potatoes	Sprinkled with domestic sewage	40 days
	Carrots	Sprinkled with domestic sewage	10 days
	Cabbage and gooseberries	Sprinkled with domestic sewage	5 days
Shigella	Streams	Not stated	30 minutes to 4 day
	Harvested Fruits	AC	Minutes to 5 days
	Market tomatoes	AC	At least 2 days
	Market apples	AC	At least 6 days
	Tomatoes	AC	2-7 days
Tubercle Bacilli	Soil	AC	6 months
	Grass	AC	14-15 months

*Artifical Contamination

in garden soil. Gudzhabidze (91) reported in the Soviet Union that Ascaris ova survived 2-5 years in soil of irrigated agriculture fields. The literature reviewed does not reveal any studies in the United States where Ascaris ova survived in sludge amended soils for more than one year.

Hess et al.(92) reported the survival of salmonellae on grass contaminated with sludge for 40 to 58 weeks in a dry atmosphere. McCarty and King (93) found that enteric pathogens could survive and remain virulent for up to two months. Rudolfs et. al. (94) concluded from field studies that the survival of representatives of the Salmonella and Shigella genera on tomato surfaces did not exceed seven days, even when the organisms were applied with fecal organic material. He attributed their short survival time to the lack of resistant stages; thus making them more vulnerable to adverse environmental conditions.

Martin (95), inoculating sterile virgin soils with E. typhosa, found they died out rapidly, but in sterilized contaminated soils growth occurred and the bacteria survived for numerous months. Rudolfs (94) in his literature review, found that the survival time of E. typhosa ranged from less than 24 hours to more than two years in freezing moist soils, but generally less than 100 days.

Approximately 90 different enteric viruses have been recovered from municipal sewage. However, there are few published reports on the survival of viruses in soil, and persistence on crops. Larkin et al. (96) described the persistence of polioviruses for 14 to 30 days on lettuce and radishes inoculated with sludge. According to Cliver (97) the soil is generally not a very adverse environment for viruses. Neither chemical nor biological inactivation occurs very rapidly, but enteroviruses do lose infectiousness as a function of time and temperature in the soil. Poliovirus 1, retained in sand from septic tank effluent, was inactivated at a rate of 13 to 18 percent per day at 20 to 25 C and at 1.1 percent per day at 6 C to 8 C. (97)

Rudolfs et al. (94) reported that unlike pathogenic bacteria, the parasitic amoeba, *Endamoeba histolytica*, forms resistant cysts which enable the organism to survive under adverse conditions. However, on the basis of laboratory and field studies on the survival of *Endamoeba histolytica* cysts, the cysts proved to be extremely sensitive to desiccation. Rudolfs concluded from his studies that field-grown crops contaminated with cysts of *E. histolytica* are considered safe in the temperate zone one week after contamination has stopped and after two weeks in wetter tropical regions.

It has been shown in the general survey of the literature (94) that certain parasite eggs, especially those of *Ascaris*, are markedly resistant to external conditions. Yoshida (98) found that mature eggs of *A. lumbriocoides* were still viable after five to six months under layers of soil in winter. He also found (99) that exposure to strong sunlight checked egg development and eventually killed them.

Brown (100) reported that the type of soil was an important factor in the viability of Ascaris eggs. Experiments showed Ascaris eggs in feces deposited on sandy soil in the sun were degenerated in 21 days. In the shade, however, 91 percent of the eggs contained mobile embryos in 35 days, and decreased to 69 percent in 54 days.

Otto (101) studied the moisture requirements of Ascaris eggs and found they did not develop to embryonation in atmosphere of less than 80 percent relative humidity, although they remained viable for varying lengths of time in atmospheres containing less moisture.

Spindler (102) in his studies on isolating Ascaris eggs from soil, found the number of embryonated eggs to be suprisingly small in spite of the fact that the soils were, in many cases, being subjected to continuous application of sewage. Vassilkova (103) in his study of contamination of sewage farm vegetables with helminth eggs, reported that the Ascaris eggs found on vegetables, only 36 percent were viable.

Except in the two reported cases (90,91) the literature indicates that the survival time of most pathogens found in wastewater sludge is limited to weeks or months, depending on environmental conditions.

3.13 Methods for Biological Examination of Solid Waste

Bacteria

Mirdza L. Peterson of EPA has published "Methods for Bacteriological Examination of Solid Waste and Waste Effluents." (104) After examining methods currently available for measuring the bacteriological quality of solid waste, reliable methods were established which are best suited to routinely measure, under practical conditions, the bacteriological quality of solid waste in and around waste processing areas. These methods were not developed to be an all-inclusive battery of tests for microorganisms in solid waste; rather, these methods test for only a few of the possible microorganisms in the solid waste.

Three procedural lines of investigation were undertaken in this effort: (1) to develop methods suitable for indicating the sanitary quality of solid waste before and after processing or disposal; (2) to develop methods suitable for determining the efficacy of operational procedures in removing or destroying the microorganisms; and, (3) to develop methods suitable for indicating the health hazard of solid waste in which pathogenic species may be present in small numbers. Methods presented in this publication are ones for determining: total viable bacterial cell number, total coliforms, fecal coliforms, heat-resistant spores, and enteric pathogens, especially Salmonella sp.

The determination of approximate total viable bacteria multiplying at a temperature of 35 C may yield useful information concerning the sanitary quality of a waste entering a processing or a disposal site, and provide useful information in judging the efficiency of procedures employed in solid waste processing and/or disposal operations.

The coliform bacteria have long been used in the United States as indicators of fecal pollution in sanitary bacteriology. Some members of the coliform group of organisms are found in the feces of warm-blooded animals, in the guts of cold-blooded animals, in soils, and on many plants. Studies have shown that warm-blooded mammal feces from humans, animals, or birds may at any time contain disease-producing microorganisms. (105) It was pointed out that cold-blooded animal feces are quantitatively insignificant as a source of pollution, but the coliform bacteria from plants or soils that have the same significance as those from feces; on the other hand, the coliform bacteria deriving from soils or plants that have not been exposed to recent fecal contamination has less public health significance.

The method for determining viable heat-resistant spore-formers is used to detect spores that survive 80 C temperature for as long as 30 minutes. With respect to survival under heat stress, most microorganisms in an actively growing (vegetative) state are readily killed by exposures to temperatures of around 70 C for 1 to 5 minutes. (106) Cells inside of material such as discarded meat products may resist heat longer because the heat does not penetrate immediately into the center of solid masses. Larger masses of non-fluid solid matter require a long exposure time (1-1/2 to 2 hr), even in an autoclave (121 C) to be heated throughly so that the center reaches a sporocidal temperature. Other reports (107) point out that although internal air temperatures of municipal incinerators usually range from 1200 to 1700 F (650 to 925 C) in continuous operation, intermittent use, overcharging of the incinerator, and high moisture content of the waste may slow the process and interfere with sterilization of the residue.

Fecal pollution of the environment by untreated and improperly disposed waste may add enteric pathogenic bacteria to a body of water or a water supply. The most common type of pathogen which may be found in untreated waste is Salmonella. The wide distribution of the many types of Salmonella in many species of animals with which man has contact or may use as food makes it difficult to prevent transmission to man. (108) Infections may occur through food, milk, or water contaminated with infected feces or urine, or by the actual ingestion of the infected animal tissues. (109) Salmonella has been found in many water supplies (110), polluted waters (111-113), raw municipal refuse and in incinerator residue (111-117)

General laboratory procedures, sample collection and preparation procedures, and bacteriological examination procedures for the organisms mentioned above can be found in Appendix A-3.1.

Parasites

The FDA has recently prepared a methodology for Ascaris determination in vegetable and sludge samples (118). The presence of Ascaris eggs, which exit from their host via the feces, is of concern to EPA in sewage sludge. These eggs are highly resistant to extreme temperatures, drying, and chemical action, and have been known to remain alive in digested sewage sludge for years. Ascaris methodology is presented in Appendix A-3.3.

Viruses

Since evidence exists that viruses can survive secondary waste treatment processes including terminal disinfection, as well as the sludge digestion process, a method for determining enteroviruses in solid waste is given. This method was developed in an EPA study entitled "Evaluation of Health Hazards Associated with Solid Waste Sewage Sludge Mixtures" (EPA contract No.68-03-0128). (55) The method was employed by the Tennessee Department of Public Health Laboratories in Nashville to determine the presence of ECHO, Coxsackie, and Polio viruses. The methodology, although given in a descriptive form in the study, has been broken down into steps in Appendix A-3.2. Since sampling procedures in the report were given for a specially prepared windrow of solid wastes, they are not included in Appendix A-3.2. Appendix A-3.1 should be consulted for sample collection procedures.

Fungi

A method for identifying pathogenic fungi in solid waste samples was developed in the same EPA report sited for virus methodology, above. (12) Again, reference should be made to Appendix A-3.1 for sampling procedures; the fungi methodology is presented in Appendix A-3.4.

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APPENDIX A-3

Methods for Biological Examination of Solid Wastes

A-3.1	Bacteriological Examination
A-3.2	Virological Examination
A-3.3	Determination of Ascaris spp. Eggs
A-3.4	Determination of Pathogenic Fungi

A-3.1

METHODS FOR BACTERIOLOGICAL EXAMINATION OF SOLID WASTE AND WASTE EFFLUENTS*

Mirdza L. Peterson

General Laboratory Procedures

Glassware washing.

All glassware known to contain infectious material must be sterilized by autoclaving before washing. All glassware that is to be used in microbiological tests must be thoroughly washed before sterilization, using a suitable detergent and hot water, and followed by hot water and distilled water rinses. Six to 12 rinses may be required to remove all traces of inhibitory residues from the glass surface.

Sterilization.

Dry heat is used for the sterilization of glass sampling bottles, foil-covered flasks, beakers, graduates, pipettes packed tightly in sealed cans, or articles that are corrosively attacked be steam. Recommended time-temperature ratio for dry heat sterilization is 170 C for 2 hr.

Saturated steam under pressure (or autoclaving) is the most frequently used sterilization method. Media, dilution water, and materials (rubber, paper, cotton, cork, heat-stable plastic tubes, and closures, for example) are sterilized by autoclaving at 121 C. Sterilization time for media and dilution water (for volumes up to 500 ml) is 15 min; 1,000-ml quantities are held for 20 min, instruments for 15 min, gloves for 20 min, and packs for 30 min (measured from the time the autoclave temperature reaches 121 C).

Membrane filters are sterilized for 10 min at 121 C with fast steam exhaust at the end of the sterilization process.

Heat-sensitive carbohydrates and other compounds are sterilized by passage through a cellulose esther membrane or another bacteria-retaining filter.

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*From Physical, Chemical, and Microbiological Methods of Solid Waste Testing. U.S. EPA Office of Testing and Monitoring, National Environmental Research Center, Cincinnati. May 1973.

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Culture media.

The use of dehydrated media is recommended whenever possible, since these products offer the advantages of good consistency from lot to lot, require less labor in preparation, and are more economical. Each lot should be tested for performance before use.

Measurement of the final pH of a prepared culture medium should be accomplished colorimetrically after autoclaving and cooling. Acceptable pH range is 7.0 ± 0.1.

Media should be stored in a cool, dry, and dark place to avoid dehydration, deterioration, and adverse light effects. Storage in the refrigerator usually prolongs the shelf-life of most media. Media should not be subjected to long periods of storage, because certain chemical reactions may occur in a medium even at refrigerator temperatures.

Many of the media referred to below can be obtained from commercial sources in a dehydrated form with complete information on their preparation. These media will therefore be listed but not described in this section. Described in this section are those media that are formulated from ingredients or from dehydrated materials. Culture media (Difco or BBL products) are listed as follows:

Bacto-agar
Bismuth sulfite agar
Blood agar
Brain heart infusion broth
Brilliant green agar
Brilliant green lactose bile, 2 percent
Coagulase mannitol agar
Dextrose
E. C. broth
Eosin methylene blue agar, Levine
Fluid thioglycollate medium
Gelatin
H-broth
Indole nitrite medium
KCN medium

Lactose
Lactose tryptose broth
Lauryl tryptose broth
Lysine decarboxylase medium
M-Endo broth
M-FC broth
MacConkey's agar
Malonate broth, Ewing modified
Maltose
Mannitol
Mannitol salt agar
Methyl red-Voges Proskauer medium
Nitrate broth
Nutrient agar
Phenol red broth base
Phosphate buffer, APHA, pH 7.2
Sabouraud's dextrose agar
Salmonella-Shigella agar
SBG enrichment broth
Selenite-F enrichment broth
SIM medium
Simmons citrate agar
Sucrose
Triple sugar iron agar
Trypticase soy agar
Tryptone glucose extract agar
Urea agar base concentrate (sterile)
XLD agar

Culture media requiring preparation.

1. Blood Agar: Suspend 40 g of trypticase soy agar in a liter of distilled water. Mix thoroughly. Heat with agitation and boil for 1 min. After solution is accomplished, sterilize by autoclaving for 15 min at 121 C. Cool agar to 45 to 50 C, and add 5 to 7 percent sterile, defibrinated sheep blood, mixing evenly throughout the medium. Pour into sterile Petri dishes. After solidification, invert dishes and incubate overnite.
2. Phenol Red Broth Base: Dissolve 15 g in a liter of distilled water. Add 5 to 10 g of desired carbohydrate. Use Durham fermentation tubes for detection of gas formation. Arrange tubes loosely in suitable containers and sterilize at 116 to 118 C for 15 min.
3. Phosphate Buffer Solution: To prepare stock phosphate buffer solution, dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml distilled water, adjust to pH 7.2 with 1N NaOH, and dilute to 1 liter with distilled water. Add 1.25 ml stock phosphate buffer solution to 1 liter distilled water. Dispense in amounts that will provide 99 ± 2.0 ml or 9 ± 0.2 ml after autoclaving 21 C for 15 min.

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COLLECTION AND PREPARATION OF SAMPLES

Method for Collection of Solid Waste or Semi-Solid Waste Samples

Equipment and materials.

Necessary items are as follows:

1. Sample containers, specimen cups, sterile, 200-ml size (Falcon Plastics, Los Angeles)
2. Sampling tongs, sterile (stainless steel, angled tips, 18 in. long)
3. Shipping container, insulated, refrigerated, 6 by 12 in. I.D.
4. Disposable gloves

Procedure.

1. Using sterile tongs, collect 20 to 40 random 100- to 200-g samples and place in sterile sampling containers. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.

2. Identify samples on tag and indicate time and date of sampling. If incinerator residue samples are taken, record operating temperatures of incinerator.

3. Deliver samples to laboratory. It is recommended that the examination be started preferably within 1 hr after collection;* the time elapsing between collection and examination should in no case exceed 8 hr.

Method for Collection of Liquid Samples-Quench and Industrial Waters or Leachate

Equipment and materials.

Necessary items include a screw-capped, 250-ml, sterile sample bottle or a 16-oz, sterile plastic bag.

Procedure.

Collect sample in bottle or plastic bag, leaving an air space in the container to facilitate mixing of the sample before examination. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.

Identify and deliver samples to laboratory. When shipping samples to laboratory, protect containers from crushing and maintain temperature below 10C during a maximum transport time of 6 hr. Examine within 2 hr. If water sample contains residual chlorine, a dechlorination agent such as sodium thiosulfate is added to collection bottles to neutralize any residual chlorine and to prevent a continuation of the bactericidal action of chlorine during the time the sample is in transit to the laboratory. Enough sodium thiosulfate is added to the clean sample bottle before sterilization to provide an approximate concentration of 100 mg per liter in the sample.

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*If sample is shipped to a laboratory for analysis and examination cannot begin within 1 hr of collection, the container must be insulated and sample maintained below 10 C during the maximum transport of 6 hr. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hr.

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Method for Collection of Incinerator Stack Effluents

Equipment and materials.

Necessary items include an Armstrong portable sampler (2), equipped with sampling assembly (Figure 1). The sampler is mounted on a steel plate (6 by 12 in.) and can be enclosed by a metal cover with a handle attached. On one side of the base is a vacuum pump with a 6-ft cord and switch. The pump is capable of drawing up to 1 cu ft per min of air (vacuum of 5.6 in. [14.3 cm] of water). On the other side of the base, a 700-ml, wide-mouth, Pyrex bottle contains 300 ml of 0.067 M phosphate buffer solution (pH 7.2) prepared by standard methods (3). The two-hole rubber stopper has a 1-in. (2.54 cm) piece of cotton-plugged glass tubing in one of the two holes. The stopper, glass tube, and contents of the bottle are maintained sterile. The bottle is held to the base plate by three removable spring clips, which are attached at the base and at a wire triangle slipped over the top of the bottle. The sampling probe is made of stainless steel tubing of appropriate diameter (e.g., 0.25-in. I.D. [0.64 cm]). The probe end has a right-angle bend so that the opening faces the stack-gas current. The tubing must be long enough to reach all parts of the stack. The tubing is coiled to permit additional cooling of the gases and is straight for 1 or 2 ft (30.48 or 60.96 cm) at a right angle to the other straight length. Before use, the sampling probe is sterilized by dry heat sterilization. It is important to keep the inside of the probe dry to minimize adsorption of microorganisms on the walls of the tubing. When sampling, the probe is inserted into the stack at locations that will yield a representative sample. The other end of the sterile probe is inserted through the sterile rubber stopper to approximately 0.5 in. (1.27 cm) above the buffered water. This is done to reduce the frothing that would occur if the probe were inserted below the surface; enough froth results in capturing the microorganisms.

Procedure.

1. Draw stack effluent through the sterile stainless steel tube by a 1.0 cfm vacuum pump; cool the tube with a water jacket.
2. Obtain a 10-cu-ft sample by drawing the stack effluent for 10 min.
3. Identify sample on tag and examine within 4 hr. The Armstrong portable sampler provides a method for qualitative, nonisokinetic sampling and is adjustable to isokinetic conditions.

Method for Collection of Dust Samples

Equipment and materials.

Necessary items include the following:

1. Andersen sampler (4)
2. Trypticase soy agar containing 5 percent sheep blood (6 plates per sample)
3. Eosin methylene blue agar

Procedure.

1. Draw air through the sterile, assembled sampler at 1.0 cfm with a vacuum of 15 in. of mercury.
2. Remove agar plates from the sampler, cover, and incubate at 35 ±0.5 C. Use aseptic technique throughout the procedure.

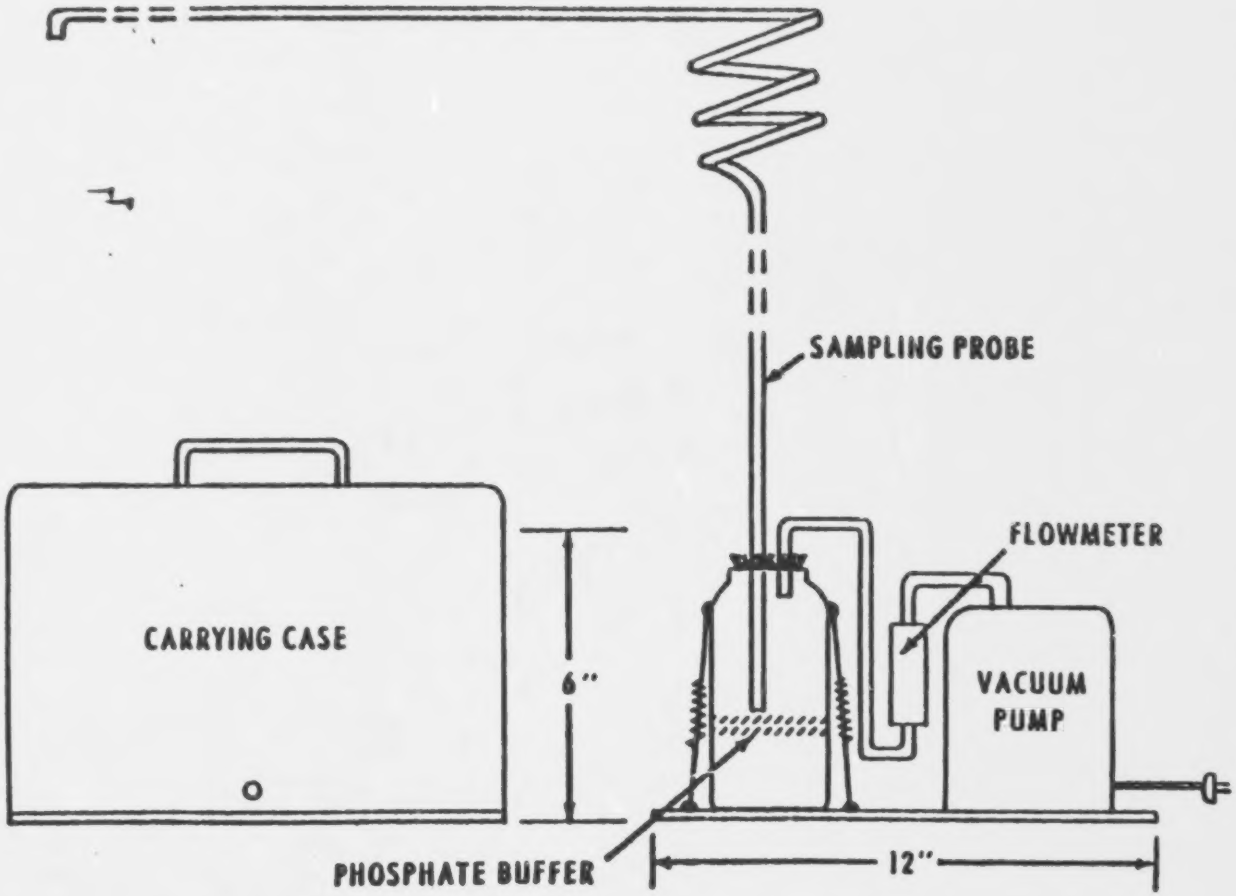
SAMPLING PROBE
FLOWMETER
CARRYING CASE
6"
VACUUM PUMP
PHOSPHATE BUFFER
12"

Figure 1. Portable sampler for microorganisms in incinerator stack emission.

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Method for Preparation of Solid and Semi-Solid Samples for Analyses

Equipment and materials.

Necessary items are as follows:

1. Cold phosphate buffer, 0.067 M, pH 7.2, sterile (3)
2. Blender, Waring (Model 1088), sterile
3. Balance, with weights, 500-g capacity
4. Tongs, sterile
5. Beakers, two, 5,000 ml and 1,000-ml sizes, sterile, covered with aluminum foil before sterilization.

Procedure.

1. Using aseptic technique, composite all random samples into a 5,000-ml beaker. Mix well.
2. Weigh 200 g of the subsample into a 1,000-ml beaker.
3. Transfer the weighed sample to a sterile blender.
4. Add 1,800 ml of sterile, phosphate buffered solution to the blender.
5. Homogenize for 15 sec at 17,000 rpm (5).
6. Prepare a series of decimal dilutions as described below in "Methods for Preparation of Decimal Dilutions of a Solid, Semi-Solid, or Liquid Waste Material."

Solid waste and residue samples for enteric pathogenic bacteria are examined directly without homog-tation.

BACTERIOLOGICAL EXAMINATION OF WASTE AND RELATED MATERIALS

Method for Preparation of Decimal Dilutions of a Solid, Semi-Solid, or Liquid Waste Material

Immediately after homogenization of any sample (see procedure under Method for Preparation of Solid and Semi-Solid Samples for Analyses) transfer a 1-ml portion of the homogenate (10^{-1} dil) to a dilution bottle containing 99 ml of phosphate buffered solution. Stopper and shake the bottle 25 times.

Prepare dilutions as indicated in Figure 2. Again shake each dilution vigorously 25 times after adding an aliquot of sample.

These dilutions are used to inoculate a series of selected culture media for the detection of various groups of microorganisms as described in the following sections of this paper.

Methods for Total Viable Bacterial Cell Number

The chief cultural method for determining total viable bacterial densities has been the agar plate method (3, 6, 7). Experience indicates that an enumeration of total number of viable bacteria multiplying at a temperature of 35 C may yield useful information concerning the sanitary quality of the waste entering a processing or a disposal site and provide useful information in judging the efficiency of procedures used in solid waste processing and/or disposal operations. The viable microbial count also provides valuable information concerning the microbiological quality of ironmental aerosols existing in or around a waste processing plant or a disposal site.

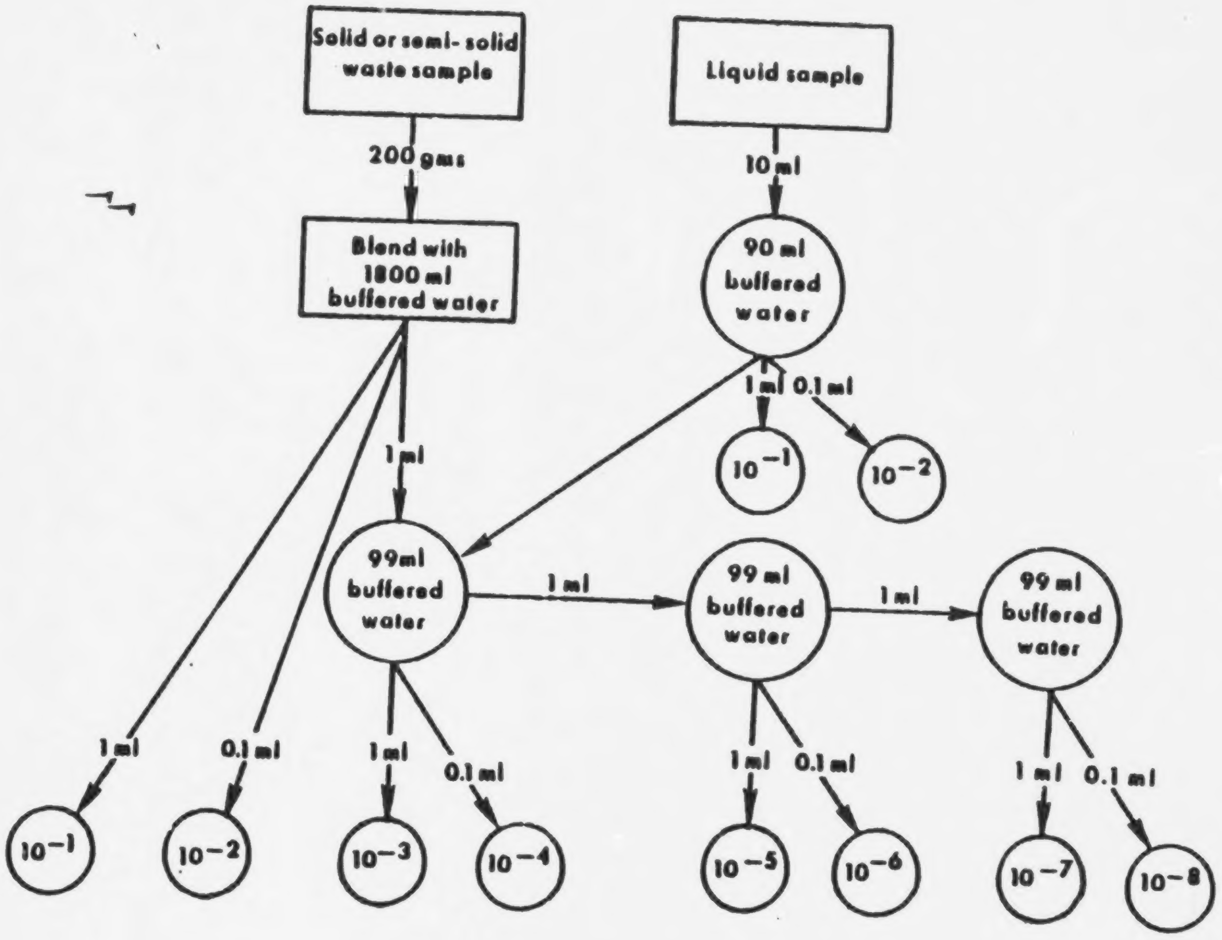
Solid or semi-solid waste sample
Liquid sample
200 gms
10 ml
Blend with 1800 ml buffered water
90 ml buffered water
1 ml
0.1 ml
10^{-1}
10^{-2}
1 ml
99ml buffered water
1 ml
99 ml buffered water
1 ml
99 ml buffered water
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}

Figure 2. Preparation of decimal dilutions.

Equipment, materials, and culture media.

1. Pipettes, 1.1 ml with 0.1 ml and 1 ml graduations
2. Dilution blanks, phosphate buffered solution, 99 ml ± 1 ml (cold)
3. Culture dishes (100 x 15 mm), plastic, sterile
4. Water bath for tempering agar, 45 ± 1 C
5. Incubator 35 ± 0.5 C
6. Colony counter, Quebeck
7. Sterile glass spreader, bent rod
8. Trypticase soy agar with 7 percent defibrinated sheep blood (TSA + blood)
9. Tryptone glucose extract agar (TGE)

Prepare TGE agar as indicated on label and hold in a melted condition in the water bath (45 C).

Dissolve ingredients of TSA and heat to boiling. Sterilize by autoclaving at 121 C for 15 min. Cool to 45 C and add sheep blood. Dispense in Petri plates and allow to solidify. Invert plates and place them in incubator overnight to dry.

ocedure for bacterial count by pour plate.

. Pipette 1 ml, 0.1 ml, or other suitable volume of the sample into each of appropriately marked, duplicate culture plates, being sure to shake each dilution bottle vigorously 25 times to resuspend material that may have settled out.

2. Add 10 to 12 ml of melted TGE agar to the sample in the Petri plate.
3. Mix dilution and the agar medium by rotating or tilting the plate.
4. Allow plates to solidify as rapidly as possible after pouring.
5. Invert plates and incubate them at 35 C ± 0.5 C for 24 ± 2 hr.
6. Count all colonies using Quebeck colony counter, the objective being to count plates with 30 to 300 colonies.
7. Compute the colony count per gram of waste (wet weight) or related solid material, and per 100 ml of water. The number of bacteria should not include more than two significant figures.

Procedure for bacterial count by streak plate.

1. Dispense 0.1 ml samples of the serially diluted homogenate (or liquid) on the surface of each of appropriately marked, duplicate TSA + blood agar plates.
2. Using a sterile glass spreader and starting with the highest dilution plates, spread the inoculum evenly over the agar surface.
3. Invert plates and incubate them at 35 C for 24 hr ± 2 hr.
4. Count the number of colonies on plates with 30 to 300 colonies.
5. Select and mark colonies for further testing.

Methods for Presence of Members of Coliform Group

The presence of fecal matter in waste and related materials is determined by the standard tests for the coliform group described in Standard Methods for the Examination of Water and Waste Water (3). The completed Most Probable Number (MPN) procedure is employed. The testing method includes the elevated temperature test (44.5 C) that indicates the fecal or nonfecal origin of coliform bacteria. Comparative laboratory studies conducted showed that the MPN estimate is the most suitable method for achieving a representative enumeration of the coliform organisms in solid waste and waste effluents (9).

Equipment and materials.

1. Pipettes, sterile–deliveries to 10 ml, 1 ml (1.1 ml), and 0.1 ml
2. Media prepared in fermentation tubes:
    Lauryl tryptose broth
    Brilliant green lactose bile broth, 2 percent
    Lactose tryptose broth
    E.C. broth
3. Media for plating:
    Eosin methylene blue agar plates
    Nutrient agar slants
4. Dilution blanks, phosphate buffer solution, sterile, 99-ml or 90-ml amounts
5. Incubator, adjusted to 35 C ± 0.5 C
6. Water bath, adjusted to 44.5 C ± 0.2 C

Procedure for total coliform group.

Presumptive Test.

1. Inoculate a predetermined volume of sample into each of 5 lauryl tryptose broth tubes. The portions of the sample used for inoculation should be decimal multiples and submultiples of 1 ml.
2. Incubate the fermentation tubes at 35 ± 0.5 C for 24 ± 2 hr.
3. Examine for the presence of gas. If no gas is formed, incubate up to 48 ± 3 hr. Record the presence or absence of gas formation at each examination of the tubes, regardless of the amount.

Confirmed Test.

1. Submit all presumptive test tubes showing any amount of gas at the end of 24- and 48-hr incubation to the confirmed test. Using a sterile platinum loop 3 mm in diameter, transfer one loopful of medium from the presumptive test fermentation tube to a fermentation tube containing brilliant green lactose bile broth.
2. Incubate the inoculated brilliant green lactose bile broth tube for 48 ±3 hr at 35 ± 0.5 C. The presence of gas in any amount in the fermentation tube of the brilliant green lactose bile broth within 48 ± 3 hr indicates a positive confirmed test.

Completed Test.

1. Submit all confirmed test tubes showing any amount of gas to the completed test. Streak an eosin methylene blue agar plate from each brilliant green bile broth tube as soon as possible after the appearance of gas.

2. Incubate the plates at 35 ± 0.5 C for 24 ± 2 hr.

3. Fish one or more typical or atypical colonies from plating medium to lactose tryptose broth fermentation tubes and nutrient agar slants.

4. Incubate the broth tubes and the agar slants at 35 ± 0.5 C for 24 ± 2 hr or 48 ± 3 hr if gas is not produced in 24 hr.

5. Prepare gram stained smears from the nutrient agar slants if gas is produced in any amount from lactose broth.

6. Examine smears under oil immersion. If typical coliform staining and morphology are found on the slant, the test may be considered completed and the presence of coliform organisms demonstrated.

Procedure for fecal coliform group (E. C. broth).

1. Submit all gas positive tubes from the Standard Methods presumptive test (lauryl tryptose broth) to the fecal coliform test. Inoculate an E. C. broth fermentation tube with a 3-mm loop of broth from a positive presumptive tube.

2. Incubate the broth tube in a water bath at 44.5 ± 0.2 C for 24 hr. All E. C. tubes must be placed in the water bath within 30 min after planting.

3. Gas production in the E. C. broth fermentation tubes within 24 hr ± 2 hr is considered a positive reaction indicating fecal origin.

Computing and recording most probable number (MPN).

The calculated estimate and the 95 percent confidence limits of the MPN described in the 13th edition of Standards Methods for Examination of Water and Waste Water (3) are presented in Table 1. This table is based on five 10-ml, five 1.0-ml, and five 0.1-ml sample portions. When the series of decimal dilutions such as 1.0, 0.1, and 0.01 ml are planted, record 10 times the value in the table; if a combination of portions of 0.1, 0.01, and 0.001 ml are planted, record 100 times the value in the table. MPN values for solid samples are calculated per g of wet weight; MPN for liquid samples are recorded per 100 ml.

Method to Determine the Presence of Viable Heat-Resistant Spore Number

Equipment and materials.

1. Test tubes, sterile, screw capped, 20 x 150 mm
2. Pipettes, sterile, graduated, 10-ml
3. Water bath, electrically heated, thermostatically controlled at 80 ± 0.5 C, equipped with thermometer (range 0 to 110 C), NBS certified. Volume of water should be sufficient to absorb cooling effect of rack of tubes without drop in temperature greater than 0.5 C.
4. Test tube support for holding tubes

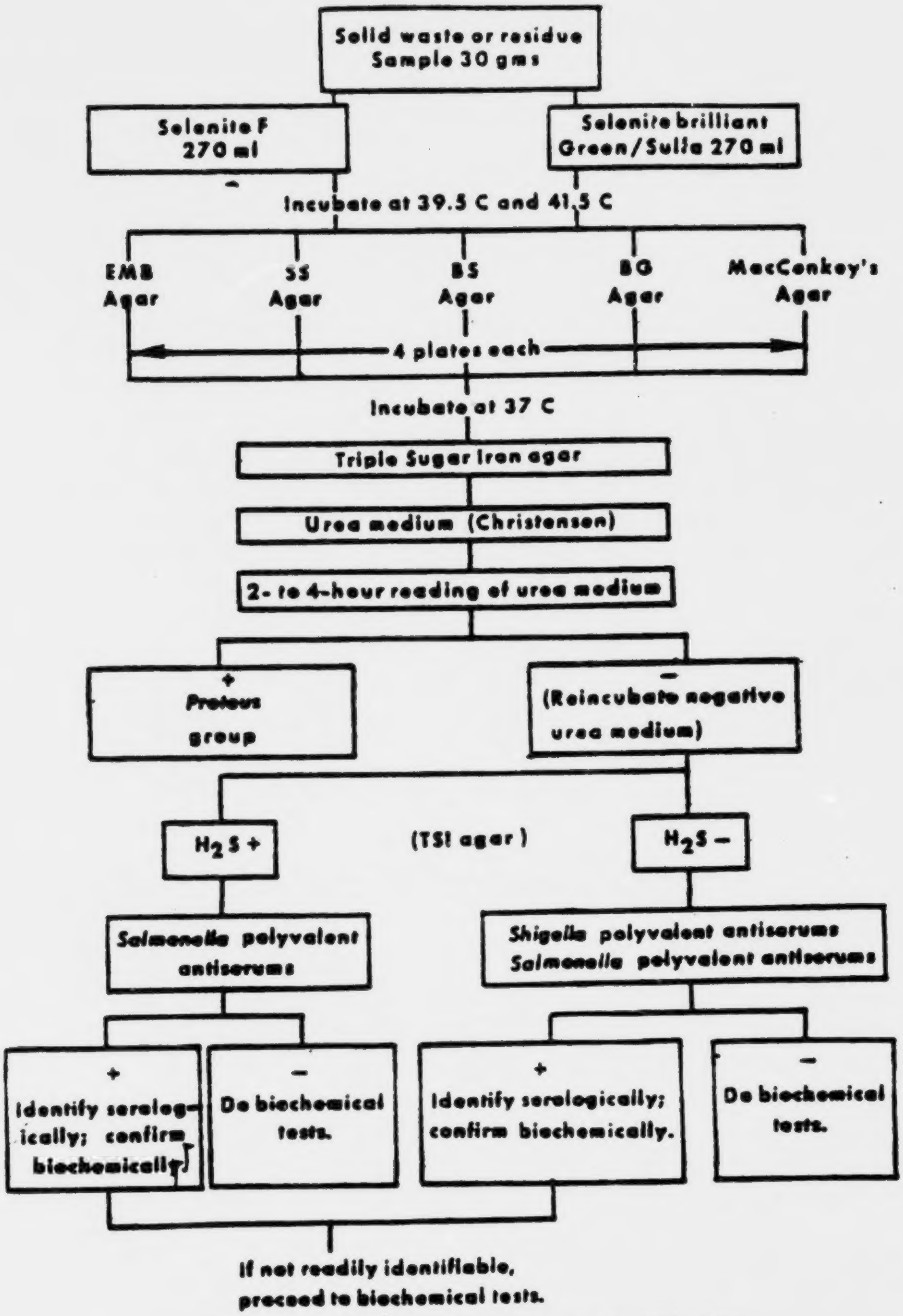
Solid waste or residue Sample 30 gms
Selenite F 270 ml
Selenite brilliant Green/Sulfa 270 ml
Incubate at 39.5 C and 41.5 C
EMB Agar
SS Agar
BS Agar
BG Agar
MacConkey's Agar
4 plates each
Incubate at 37 C
Triple Sugar Iron agar
Urea medium (Christensen)
2- to 4-hour reading of urea medium
+
Proteus group
−
(Reincubate negative urea medium)
H_2S +
(TSI agar)
H_2S −
Salmonella polyvalent antiserums
Shigella polyvalent antiserums
Salmonella polyvalent antiserums
+
Identify serologically; confirm biochemically.
−
Do biochemical tests.
+
Identify serologically; confirm biochemically.
−
Do biochemical tests.
If not readily identifiable, proceed to biochemical tests.

Figure 3. Isolation and preliminary identification.

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Procedure.

1. Transfer 10 ml from each original sample and from each successive dilution thereof to screw-capped test tubes, being careful to avoid contaminating the lip and upper portion of tube with sample.
2. Place tubes in a rack.
3. Place rack of tubes in water bath at 80 C for 30 min. Tubes should be immersed so that the water line is approximately 1½ in. above the level of samples in the tubes.
4. At the end of the 30-min holding period, remove the rack of tubes from the water bath and place in cold water for 5 min to cool.
5. Determine viable heat-resistant spore count by agar pour-plate method (see, Procedure for Bacterial Count by Pour Plate *under* Methods for Total Viable Bacterial Cell Number).
6. Report results as "viable heat-resistant spore count per gram."

Methods to Detect Enteric Pathogenic Bacteria

Equipment, materials and media.

1. Incubator, 37 C
2. Water baths, constant temperature, 39.5 C and 41.5 C
Flasks, wide-mouth, 500-ml
Membrane filter holder
Flasks, vacuum, 2,000-ml
6. Balance, with weights, 100-g capacity
7. Needle, inoculating
8. Media and reagents:
    - Selenite brilliant green/sulfa enrichment broth
    - Selenite F enrichment broth
    - Eosin methylene blue (EMB) agar
    - Salmonella-Shigella (SS) agar
    - Bismuth sulfite (BS) agar
    - McConkey's agar
    - Brilliant green (BG) agar
    - Triple sugar iron (TSI) agar
    - Urea medium
    - XLD agar
    - Salmonella antiserums
    - Shigella antiserums
    - Biochemical media (15)
9. Diatomaceous earth (Johns-Manville, Celite 505), sterile

Procedure to detect pathogens in solid waste and incinerator residue.

1. Add a previously weighed, 30-g sample to each of two flasks containing 270 ml Selenite F enrichment broth, and also to each of two flasks containing 270 ml Selenite brilliant green/sulfa (SBG) enrichment broth. Shake to mix.

2. Incubate one Selenite F and one SBG flask at 39.5 C and the other two at 41.5 C for 16 to 18 hr.

3. After incubation, streak one loopful from each enrichment medium on each of four plates of Salmonella-Shigella and other selective enteric media.
4. Incubate the plates at 37 C for 24 to 48 hr and pick suspicious colonies to triple sugar iron agar slants.
5. Incubate the slants at 37 C for 24 hr and complete identification by appropriate methods as described by Edwards and Ewing (20). Isolation, preliminary identification, and biochemical testing are described in Figure 3 and in Table 2.

Procedure to detect pathogens in quench or industrial waters and in leachate.

1. Place enough sterile diatomaceous earth on the screen of a stainless steel membrane filter holder to form a 1-in. layer.
2. Filter 800-ml sample through the earth layer.
3. Remove one-half the diatomaceous earth layer with a sterile spatula and place into 90 ml of Selenite F enrichment broth; place other half of the earth layer into 90 ml of Selenite brilliant green/sulfa enrichment broth. Shake both flasks to mix.
4. Incubate both flasks in a water bath at 39.5 C for 16 to 18 hr.
5. Proceed as directed in steps 3 through 5 of Procedure to Select Pathogens in Solid Waste and Incinerator Residue.

Method for Examination of Stack Effluents

As described in Methods for Collection of Incinerator Stack Effluents (using the Armstrong sampler), the microorganisms are impinged into a 300-ml phosphate buffer solution.

Filter 100 ml of the "inoculated" phosphate buffer solution through a 0.45μ HA membrane ter (3).
2. Transfer membrane filter with sterile forceps to a culture plate containing trypticase soy agar.
3. Incubate culture plate under constant saturated humidity for 20 hr (± 2 hr) at 35 C.
4. After incubation, remove cover from culture plate and determine colony count with the aid of a low-power (10-15 magnifications) binocular, wide-field microscope. Characterize colonies using specific isolation media.
5. Remove a 10-ml portion of the "inoculated" phosphate buffer solution and examine for viable heat-resistant spores as directed in steps 1 through 6 of the procedure under Method to Determine the Presence of Viable Heat-Resistant Spore Numbers.

Microbial counts are reported as organisms per cubic foot of air. If the sample is not taken under isokinetic conditions, the results are qualitative. If the stack velocity is known and remains relatively constant, however, the flow rate of the sampler can be adjusted to isokinetic conditions to yield quantitative results.

Method for Examination of Dust

As described in Methods for Collection of Dust Samples, the Andersen sampler is used with two types of media–trypticase soy agar (TSA-BBL product) containing 5 percent sheep blood, and eosin methylene blue agar (EMB-Difco product). The TSA/blood agar is used to isolate a wider range of fastidious organisms such as *Staphylococci*, *Streptococci*, and *Diplococci*. The EMB agar is used to isolate gram-negative bacteria. The plates are incubated aerobically at 37 C for 24 hr. (Preliminary studies showed that few organisms in the dust would grow under anaerobic conditions.) Enumeration of colonies is made with a Quebec colony counter. Microbial count is reported as organisms per bic foot of air. At times, when microbial counts are high, the sampling time is 0.25 min, thus yield- 0.25 cu ft air.

TABLE 2. DIFFERENTIATION OF ENTEROBACTERIACEAE BY BIOCHEMICAL TESTS.

TEST or SUBSTRATE	ESCHERICHIEAE		EDWARDSIELLEAE	SALMONELLEAE			KLEBSIELLEAE									PROTEEAE					
								Enterobacter						*Serratia*	*Pectobacterium*	*Proteus*				*Providencia*	
										hafniae		*liquefaciens*									
	Escherichia	*Shigella*	*Edwardsiella*	*Salmonella*	*Arizona*	*Citrobacter*	*Klebsiella*	*cloacae*	*aerogenes*	22C	37C	22C	37C		22C	*vulgaris*	*mirabilis*	*morganii*	*rettgeri*	*alcalifaciens*	*stuartii*
INDOL	+	- or +	+	-	-	-	- or +	-	-	-	-	-	-	-	- or +	+	-	+	+	+	+
METHYL RED	+	+	+	+	+	+	-	-	-	+ or -	-	+ or -	- or +	- or +	+ or -	+	+	+	+	+	+
VOGES - PROSKAUER	-	-	-	-	-	-	+	+	+	+ or -	+	- or +	+ or -	+	- or +	-	- or +	-	-	-	-
SIMMONS'S CITRATE	-	-	-	d	+	+	+	+	+	(+) or -	d	+	+	+	d	d	+ or (+)	-	+	+	+
HYDROGEN SULFIDE (TSI)	-	-	+	+	+	+ or -	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
UREASE	-	-	-	-	-	d^w	+	+ or -	-	-	-	d	-	d^w	d^w	+	+	+	+	-	-
KCN	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+ or -	+	+	+	+	+	+
MOTILITY	+ or -	-	+	+	+	+	-	+	+	+	+	d	+	+	+ or -	+	+	+	+	+	+
GELATIN (22C)	-	-	-	-	(+)	-	-	(+) or -	- or (+)		-		+	+	+ or (+)	+ or (+)	+	-	-	-	-
LYSINE DECARBOXYLASE	d	-	+	+	+	-	+	-	+	+	+	+ or -	+	+	-	-	-	-	-	-	-
ARGININE DIHYDROLASE	d	- or (+)	-	(+) or +	+ or (+)	d	-	+	-	-	-	-	-	-	- or +	-	-	-	-	-	-
ORNITHINE DECARBOXYLASE	d	d[1]	+	+	+	d	-	+	+	+	+	+	+	+	-	-	+	+	-	-	-
PHENYLALANINE DEAMINASE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
MALONATE	-	-	-	-	+	d	+	+ or -	+ or -	+ or -	+ or -	-	-	-	- or +	-	-	-	-	-	-
GAS FROM GLUCOSE	+	-[1]	+	+	+	+	+	+	+	+	+	+	+	+ or -[3]	- or +	+ or -	+	d	- or +	+ or -	-
LACTOSE	+	-[1]	-	-	d	d	+	+	+	- or (+)	- or (+)	d	(+)	- or (+)	d	-	-	-	-	-	-
SUCROSE	d	-[1]	-	-	-	d	+	+	+	d	d	+	+	+	+	+	d	-	d	d	d
MANNITOL	+	+ or -	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+ or -	-	d
DULCITOL	d	d	-	d[2]	-	d	- or +	- or +	-	-	-	-	-	-	-	-	-	-	-	-	-
SALICIN	d	-	-	-	-	d	+	+ or (+)	+	d	d	+	+	+	+	d	d	-	d	-	-
ADONITOL	-	-	-	-	-	-	+ or -	- or +	+	-	-	d	d	d	-	-	-	-	d	+	-
INOSITOL	-	-	-	d	-	-	+	d	+	-	-	+	+	d	-	-	-	-	+	-	+
SORBITOL	+	d	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	d	-	d
ARABINOSE	+	d	-	+[2]	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
RAFFINOSE	d	d	-	-	-	d	+	+	+	-	-	+	+	-	+ or (+)	-	-	-	-	-	-
RHAMNOSE	d	d	-	+	+	+	+	+	+	+	+	-	-	-	d	-	-	-	+ or -	-	-

(1) Certain bioserotypes of S. flexneri produce gas. S. sonnei cultures ferment lactose and sucrose slowly and decarboxylate ornithine.
(2) S. typhi, S. cholerae-suis, S. enteritidis bioser. Paratyphi A and Pullorum, and a few others ordinarily do not ferment dulcitol promptly. S. cholerae-suis does not ferment arabinose.
(3) Gas volumes produced by cultures of Serratia, Proteus, and Providencia are small.

+, 90 percent or more positive in 1 or 2 days. -, 90 percent or more negative. d, different biochemical types [+, (+), -]. (+), delayed positive. + or -, majority of cultures positive. - or +, majority negative. w, weakly positive reaction.

*Source: *Identification of Enterobacteriaceae* by P.R. Edwards and W.H. Ewing. Third edition, 1972, p. 24. Reproduced by permission,
[illegible] Minneapolis, Minnesota

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A-3.2 METHOD for VIROLOGICAL EXAMINATION of SOLID WASTE

1. Place 2 g of sample in flask containing 20 ml of cold, sterile distilled water and glass beads.

2. Vigorously shake flask.

3. Pour contents into sterile centrifuge tube.

4. Clarify suspension by centrifugation in a refrigerated centrifuge (4 C) at 1500 rpm for 20 minutes.

5. Pour off supernate and recentrifuge for 1 hr. at 3000 rpm.

6. Remove clear supernate from sediment.

7. Add an antibiotic solution to give a final concentration per ml of 1000 units of penicillin and 1000 ug of streptomycin.

8. Hold sample at room temperature for 30 min.

9. Inoculate sample into 3 tubes of primary monkey kidney cells (e.g. African Green).

10. Inoculate sample also into 3 tubes of Hep 2 cells.

11. Incubate tubes in roller drum at 98.6 F (37 C) for 8 to 9 days.

12. Observe cell cultures daily for virus activity.

A-3.3 DETERMINATION OF ASCARIS spp. EGGS in SOLID WASTE

1. Materials

1.1 Balance: 10 g - 1 kg capacity.

1.2 Beakers: 150 ml & 600 ml

1.3 Bottle: 125 ml, Wheaton.

1.4 Bottle shaker.

1.5 Brush: B-8695 Scientific Products.

1.6 Centrifuge: rotor radius 14.6 cm.

1.7 Centrifuge tubes: 15 ml and 50 ml.

1.8 Cheesecloth: FSN 8305-00-205-3496.

1.9 Counter: differential

1.10 Culture dish: with 2 mm grid.

1.11 Inverted microscope

1.12 Pipettes: Pasteur type and 5 ml serological.

1.13 Rubber bulb: ca. 2 ml

1.14 Tray: round, 10.5 inches diameter, 3 inches high e.g., Beckman Instrument Co. 82-018.

2. Reagents

2.1 Saline: 0.85% NaCl in H_2O.

2.2 Nacconol: 0.4% of concentrate in H_2O

2.3 Hydrochloric acid: 2% solution in H_2O.

2.4 Solvent: alcohol:acetone:xylene in 1:1:2 ratios.

3. Sample Preparation

3.1 Vegetable Samples

3.1.1 The sample size for vegetables is 1 kg. Leafy vegetables occuring in heads (cabbage, lettuce etc.) are first separated into individual leaves.

3.1.2 Dispense 250 ml of the nacconol solution into the tray.

3.1.3 Individual vegetables are placed in the tray, and thoroughly scrubbed with the brush.

3.1.4 Allow the vegetable to drain for 10 seconds and then set aside.

3.1.5 Steps 3.1.2 & 3.1.3 are repeated until the entire sample is washed; nacconol solution is replaced as necessary.

3.1.6 Pour the contents of the tray into a 600 ml beaker.

3.1.7 Rinse the tray 3 times with 25 ml of the nacconol solution and add each rinse to the beaker.

3.1.8 Distribute the suspension into 50 ml centrifuge tubes.

3.1.9 Rinse the beaker 3 times with 10 ml nacconol solution, then add each rinse to the centrifuge tubes or to an additional centrifuge tube.

3.2 Sludge Samples

3.2.1 Weight out 10 g of sludge and add it to 90 ml of saline in the 125 ml bottle.

3.2.2 Place the bottle on the shaker; shake vigorously for 5 minutes (the speed control of an International Size-2 Shaker (International Equipment Company, Model 2) is set at the midpoint).

3.2.3 Pour the suspension through 1 layer of wet cheesecloth into a 150 ml beaker.

3.2.4 Rinse the bottle 3 times with 5 ml saline and add each rinse to the beaker.

3.2.5 Transfer the contents of the beaker to seven 15 ml centrifuge tubes.

3.2.6 Rinse the beaker 3 times with 5 ml of saline and add each rinse to the centrifuge tubes.

4. Centrifugation Procedure

4.1 Centrifuge the tubes collected in 3.1 and/or 3.2 at 2,000 rpm (radius 14.6 cm) for 4 minutes.

4.2 Remove and discard the supernatant.

4.3 Add 2 ml of saline to each tube.

4.4 Combine the sediments into one tube using a Pasteur pipette to transfer the sediment and to rinse each tube 3 times with 2 ml of saline. Each rinse is also added to the collecting tube.

4.5 When the collecting tube is full, it is balanced with a blank, centrifuged at 2,000 rpm for 4 minutes; supernatant is discarded. Repeat if necessary.

4.6 Add saline to the 15 or 50 ml graduation mark on the collecting tube and resuspend the sediment; centrifuge at 2,000 rpm for 4 minutes.

4.7 Discard the supernatant; add 2 ml of saline and resuspend the sediment.

4.8 Transfer the suspension to the culture dish; rinse the tube 3 times with 2 ml of saline and add each rinse to the culture dish. Add 8 ml of the 2% hydrochloric acid to the dish (to prevent mold growth) and cover the dish.

5. A Warning

Ascaris spp. ova are infective to humans. Areas which become contaminated should be wiped with the solvent solution.

6. Viability Determination

6.1 Culture dishes from step 4.8 are allowed to incubate at room temperature (ca 24 C) for 3 weeks.

6.1 Check the fluid levels in the culture dishes twice weekly; a depth of 3 mm should be maintained by addition of H_2).

7. Microscopic Examination

7.1 Systematically search the bottom of the dish with the aid of an inverted microscope, using the grid markings as guides.

7.2 With American Optical 1810 equipment 24X objective and 10X eyepieces, the 2 mm grid width is just spanned.

7.3 Count the embryonated and the unembryonated eggs with the differential counter.

7.4 **Ascaris** spp. eggs are usually 60 to 70 um long and 40 to 50 mm wide; the outer covering, a rough albuminous coat, is often yellowish brown in color; beneath the coat there is a thick layer of clear shell. If the center of the egg is amorphous orslightly granular, the egg was not fertilized and will not develop. An organized center indicates a fertilized egg. With incubation (step 6 above), fertilized eggs develop into embryonated eggs which contain a second-stage nematode larva in a cuticular sheath. Types of Ascaris spp. eggs are illustrated in the following references.

8. References

8.1 Faust, E.C. Beaver, P.C., Jung, R.C. 1968. Animal Agents and Vectors of Human Disease. Lea and Febiger, Philadelphia.

8.2 Markell, E.K. and Voge, M. 1971. Medical Parasitology. Saunders, W.B., Philadelphia.

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A-3.4 METHODS FOR IDENTIFYING PATHOGENIC FUNGI IN SOLID WASTE

1. Sample Preparation

1.1 Add 5 g of composite sample to 100 ml of sterile physicological saline (0.85% salt solution).

1.2 Shake to suspend sample.

1.3 Separate supernatant and centrifuge at 2500 rpm for 15 min.

1.4 Decant supernatant

1.5 Thoroughly mix sediment and add to sterile screw cap vials containing 10,000 units of penicillin and 10 mg of streptomycin.

1.6 Allow suspension to stand at room temperature for 20 min.

2. Swiss Mice Inoculation

2.1 Inoculate three white Swiss mice (4 to 6 weeks of age) intraperitoneally with 0.5 ml of concentrated sediment.

2.2 At the end of 3 weeks sacrifice mice.

2.3 Remove liver and entire spleen and place in sterile petri dish.

2.4 Mince tissues.

2.5 Use small portions of minced tissues to inoculate two tubes of Sabouraud's agar and two tubes of Sabouraund's agar containing 0.5 mg of Actidione (cycloheximide) per ml and 0.05 mg of chloromycetin per liter.

2.6 Incubate cultures for 4 weeks, making weekly examinations (make smears of suspicious colonies; identify fungi by cultural characteristics.)

3. Actidione and chloromycetin inoculation

3.1 Prepare two tubes of Sabouraud's agar and two tubes of Sabouraud's agar containing 0.5 mg Actidione per ml and 0.05 g of chloromycetin per liter.

3.2 Inoculate with a small portion of concentrated sediment.

3.3 Incubate all tubes at 25 C and examine weekly.

3.4 At the end of 6 weeks make smears of suspicious colonies and identify by cultural characteristics.